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(54) Title: COMPOUNDS AND METHODS FOR THE DETECTION AND PREVENTION OF T. CRUZI INFECTION

(57) Abstract

Compounds and methods are provided for diagnosing *Trypanosoma cruzi* infection. The disclosed compounds are polypeptides, or antibodies thereto, that contain one or more epitopes of *T. cruzi* antigens. The compounds are useful in a variety of immunoassays for detecting *T. cruzi* infection. The polypeptide compounds are further useful in vaccines and pharmaceutical compositions for inducing protective immunity against Chagas' disease in individuals exposed to *T. cruzi*.

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Description

COMPOUNDS AND METHODS FOR THE DETECTION AND PREVENTION OF *T. CRUZI* INFECTION

Technical Field

The present invention relates generally to the diagnosis of *T. cruzi* infection. The invention is more particularly related to the use of one or more *T. cruzi* antigenic peptides, or antibodies thereto, in methods and diagnostic kits to screen individuals and blood supplies for *T. cruzi* infection. The invention is also directed to vaccine compositions for immunizing an individual to prevent Chagas' disease.

Background of the Invention

Protozoan parasites are a serious health threat in many areas of the world. *Trypanosoma cruzi* (*T. cruzi*) is one such parasite that infects millions of individuals, primarily in Central and South America. Infections with this parasite can cause Chagas' disease, which may result in chronic heart disease and a variety of immune system disorders. It is estimated that 18 million people in Latin America are infected with *T. cruzi*, but there is no reliable treatment for the clinical manifestations of infection. No vaccine for the prevention of Chagas' disease is currently available.

The most significant route of transmission in areas where the disease is endemic is through contact with an infected triatomid bug. In other areas, however, blood transfusions are the dominant means of transmission. To inhibit the transmission of *T. cruzi* in such regions, it is necessary to develop accurate methods for diagnosing *T. cruzi* infection in individuals and for screening blood supplies. Blood bank screening is particularly important in South America, where 0.1%-62% of samples may be infected and where the parasite is frequently transmitted by blood transfusion. There is also increasing concern that the blood supply in certain U.S. cities may be contaminated with *T. cruzi* parasites.

The diagnosis of *T. cruzi* infection has been problematic, since accurate methods for detecting the parasite that are suitable for routine use have been unavailable. During the acute phase of infection, which may last for decades, the infection may remain quiescent and the host may be asymptomatic. As a result, serological tests for *T. cruzi* infection are the most reliable and the most commonly used.

Such diagnoses are complicated, however, by the complex life cycle of the parasite and the diverse immune responses of the host. The parasite passes through

an epimastigote stage in the insect vector and two main stages in the mammalian host. One host stage is present in blood (the trypomastigote stage) and a second stage is intracellular (the amastigote stage). The multiple stages result in a diversity of antigens presented by the parasite during infection. In addition, immune responses to protozoan infection are complex, involving both humoral and cell-mediated responses to the array of parasite antigens.

While detecting antibodies against parasite antigens is the most common and reliable method of diagnosing clinical and subclinical infections, current tests are expensive and difficult. Most serological tests use whole or lysed *T. cruzi* and require positive results on two of three tests, including complement fixation, indirect immunofluorescence, passive agglutination or ELISA, to accurately detect *T. cruzi* infection. The cost and difficulty of such tests has prevented the screening of blood or sera in many endemic areas.

Accordingly, there is a need in the art for more specific and sensitive methods of detecting *T. cruzi* infections in blood supplies and individuals. The present invention fulfills these needs and further provides other related advantages.

Summary of the Invention

Briefly stated, this invention provides compounds and methods for detecting and protecting against *T. cruzi* infection in individuals and in blood supplies, and for screening for *T. cruzi* infection in biological samples. In one aspect, the present invention provides methods for detecting *T. cruzi* infection in a biological sample, comprising (a) contacting the biological sample with a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

In another aspect of this invention, polypeptides are provided comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:21, or a variant of such an antigen that differs only in conservative substitutions and/or modifications.

Within related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides diagnostic kits for detecting *T. cruzi* infection in a biological sample, comprising (a) a polypeptide

comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) a detection reagent.

In yet another aspect of the invention, methods for detecting the presence of *T. cruzi* infection in a biological sample are provided, comprising (a) contacting a biological sample with a monoclonal antibody that binds to an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) detecting in the biological sample the presence of *T. cruzi* parasites that bind to the monoclonal antibody.

Within related aspects, pharmaceutical compositions comprising the above polypeptides and a physiologically acceptable carrier, and vaccines comprising the above polypeptides in combination with an adjuvant, are also provided.

The present invention also provides, within other aspects, methods for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

Within other aspects, the present invention provides methods for detecting *T. cruzi* infection in a biological sample, comprising (a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; (b) contacting the biological sample with one or more additional polypeptides comprising one or more epitopes of other *T. cruzi* antigens, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample. In one embodiment, the additional polypeptide comprises an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications. In another embodiment, the additional polypeptides comprise an epitope of TcD (or a variant thereof that differs only in conservative substitutions and/or modifications) and an epitope of TcE (or a variant thereof that differs only in conservative substitutions and/or modifications). In yet another embodiment, the additional polypeptides comprise an epitope of TcD (or a variant thereof that differs only in conservative substitutions and/or modifications) and PEP-2 (or a variant thereof that differs only in conservative substitutions and/or modifications).

In yet further aspects, the present invention provides combination polypeptides comprising two or more polypeptides, each polypeptide comprising an epitope of a T. cruzi antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant thereof that differs only in conservative substitutions and/or modifications. Combination polypeptides comprising at least one epitope of a T. cruzi antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant thereof that differs only in conservative substitutions and/or modifications, and at least one epitope selected from the group consisting of TcD epitopes, TcE epitopes, PEP-2 epitopes and variants thereof that differ only in conservative substitutions and/or modifications are also provided.

In related aspects, methods are provided for detecting T. cruzi infection in a biological sample, comprising (a) contacting the biological sample with at least one of the above combination polypeptides and (b) detecting in the biological sample the presence of antibodies that bind to the combination polypeptide.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figure 1 is a graph comparing the reactivity of *T. cruzi* lysate and a representative polypeptide of the present invention (rTcc6) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. The bars represent ± 1 standard deviation.

Figure 2 is a graph presenting a comparison of the reactivity of representative polypeptides of the subject invention in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. Experiment 1 shows a comparison of rTcc22 and the peptides Tcc22-1 and Tcc22-1+; Experiment 2 shows a comparison of rTcc22, rTcHi12 and the peptides Tcc22-1, Tcc22-1+ and Tcc22-2.1. The bars represent ± 1 standard deviation.

Figure 3 is a graph depicting a comparison of the reactivity of *T. cruzi* lysate and a representative polypeptide (Tcc38) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals, as well as using sera from individuals with visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), tuberculosis (TB) and malaria. The bars represent ± 1 standard deviation.

Figure 4 is a graph presenting a comparison of the reactivity of *T. cruzi* lysate and several polypeptides of the present invention, representing different reading frames of the TcLo1 and TcHi10 antigens, in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. The bars represent ± 1 standard deviation.

Figure 5 is a graph comparing the reactivity of *T. cruzi* lysate and a representative polypeptide (TccLo1.2) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals, as well as sera from individuals with visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), malaria and tuberculosis (TB).

Figure 6 is a graph depicting the ELISA reactivity of a series of polypeptide combinations with *T. cruzi* positive and negative sera.

Figure 7 is a graph presenting the ELISA reactivity of a series of TcE polypeptide variants with *T. cruzi* positive and negative sera.

Figure 8 is a graph comparing the ELISA reactivity of two dipeptides, a tripeptide and a tetrapeptide of the present invention with *T. cruzi* positive and negative sera.

Figure 9 is a graph presenting the ELISA reactivity of a representative polypeptide of the present invention (TcHi29) and of TcE with sera from normal individuals, *T. cruzi* patients, and patients with other diseases.

Figure 10 is a graph comparing the ELISA reactivity of two representative dipeptide mixtures with *T. cruzi* positive and negative sera, one mixture including a TcE epitope and the other including aTcHi29 epitope of the present invention.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compounds and methods for detecting and protecting against *T. cruzi* infection in individuals and in blood supplies. The compounds of this invention generally comprise one or more epitopes of *T. cruzi* antigens. In particular, polypeptides comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22are preferred. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length (*i.e.*, native) antigens. Thus, a polypeptide comprising an epitope may consist entirely of the epitope or may contain additional sequences. The additional sequences may be derived from the native antigen or may be heterologous, and such sequences may (but

need not) be antigenic. A protein "having" a particular amino acid sequence is a protein that contains, within its full length sequence, the recited sequence. Such a protein may, or may not, contain additional amino acid sequence. The use of one or more epitopes from additional *T. cruzi* proteins, prior to or in combination with one or more epitopes of sequences recited herein, to enhance the sensitivity and specificity of the diagnosis, is also contemplated.

An "epitope," as used herein, is a portion of a *T. cruzi* antigen that reacts with sera from *T. cruzi*-infected individuals (*i.e.*, an epitope is specifically bound by one or more antibodies within such sera). Epitopes of the antigens described in the present application may generally be identified using methods known to those of ordinary skill in the art, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. For example, a polypeptide derived from a native *T. cruzi* antigen may be screened for the ability to react with pooled sera obtained from *T. cruzi*-infected patients. Suitable assays for evaluating reactivity with *T. cruzi*-infected sera, such as an enzyme linked immunosorbent assay (ELISA), are described in more detail below, and in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. An epitope of a polypeptide is a portion that reacts with such antisera at a level that is substantially similar to the reactivity of the full length polypeptide. In other words, an epitope may generate at least about 80%, and preferably at least about 100%, of the response generated by the full length polypeptide in an antibody binding assay (*e.g.*, an ELISA).

The compounds and methods of this invention also encompass variants of the above polypeptides. As used herein, a "variant" is a polypeptide that differs from the recited polypeptide only in conservative substitutions or modifications, such that it retains the antigenic properties of the recited polypeptide. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. Variants may also, or alternatively, contain other conservative modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, the polypeptide may be conjugated to a linker or other sequence for ease of synthesis or to enhance binding of the polypeptide to a solid support.

In a related aspect, combination polypeptides comprising epitopes of multiple *T. cruzi* antigens are disclosed. A "combination polypeptide" is a polypeptide in which epitopes of different *T. cruzi* antigens, or variants thereof, are joined, for example through a peptide linkage, into a single amino acid chain. The amino acid chain thus formed may be either linear or branched. The epitopes may be joined directly (*i.e.*, with no intervening amino acids) or may be joined by way of a linker sequence (*e.g.*, Gly-Cys-Gly) that does not significantly alter the antigenic properties of the epitopes. The peptide epitopes may also be linked through non-peptide linkages, such as hetero- or homo-bifunctional agents that chemically or photochemically couple between specific functional groups on the peptide epitopes such as through amino, carboxyl, or sulfhydryl groups. Bifunctional agents which may be usefully employed in the combination polypeptides of the present invention are well known to those of skill in the art. Epitopes may also be linked by means of a complementary ligand/anti-ligand pair, such as avidin/biotin, with one or more epitopes being linked to a first member of the ligand/anti-ligand pair and then being bound to the complementary member of the ligand/anti-ligand pair either in solution or in solid phase. A combination polypeptide may contain multiple epitopes of polypeptides as described herein and/or may contain epitopes of one or more other *T. cruzi* antigens, such as TcD, TcE or PEP-2, linked to an epitope described herein.

In general, *T. cruzi* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, a *T. cruzi* cDNA or genomic DNA expression library may be screened with pools of sera from *T. cruzi*-infected individuals. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989. Briefly, the bacteriophage library may be plated and transferred to filters. The filters may then be incubated with serum and a detection reagent. In the context of this invention, a "detection reagent" is any compound capable of binding to the antibody-antigen complex, which may then be detected by any of a variety of means known to those of ordinary skill in the art. Typical detection reagents for screening purposes contain a "binding agent," such as Protein A, Protein G, IgG or a lectin, coupled to a reporter group. Preferred reporter groups include, but are not limited to, enzymes, substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. More preferably, the reporter group is horseradish peroxidase, which may be detected by incubation with a substrate such as tetramethylbenzidine or 2,2'-azino-di-3-ethylbenzthiazoline sulfonic acid. Plaques containing cDNAs that express a protein that binds to an antibody in the serum may be

isolated and purified by techniques known to those of ordinary skill in the art. Appropriate methods may be found, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989.

DNA molecules having the nucleotide sequences recited in SEQ ID NO:1 - SEQ ID NO:18 may be isolated by screening a *T. cruzi* genomic expression library with pools of sera from *T. cruzi*-infected individuals, as described above. More specifically, DNA molecules having the nucleotide sequences recited in SEQ ID NO:1 - SEQ ID NO:16 may be isolated by screening the library with a pool of sera that displays serological reactivity (in an ELISA or Western assay) with parasite lysate and/or one or both of the *T. cruzi* antigens TcD and TcE, described in U.S. Patent No. 5,304,371 and U.S. Serial No. 08/403,379, filed March 14, 1995. A subsequent screen is then performed with patient sera lacking detectable anti-TcD antibody. A DNA molecule having the nucleotide sequences recited in SEQ ID NO:17 (5' end) and SEQ ID NO:18 (3' end) may be isolated by screening the genomic expression library with a pool of sera that displays lower serological reactivity (*i.e.*, detects a signal less than 3 standard deviations over background reactivity in an ELISA or Western assay) with lysate, TcD and TcE, followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody.

DNA molecules having the sequences recited in SEQ ID NO:19 - SEQ ID NO:22 may be obtained by screening an unamplified *T. cruzi* cDNA expression library with sera (both higher and lower serological reactivity) from *T. cruzi*-infected individuals, as described above.

Alternatively, DNA molecules having the sequences recited in SEQ ID NO:1 - SEQ ID NO:22 may be amplified from *T. cruzi* genomic DNA or cDNA via polymerase chain reaction. For this approach, sequence-specific primers may be designed based on the sequences provided in SEQ ID NO:1 - SEQ ID NO:22, and may be purchased or synthesized. An amplified portion of the DNA sequences may then be used to isolate the full length genomic or cDNA clones using well known techniques, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1989).

Epitopes of antigens having amino acid sequences encoded by the above DNA sequences may generally be identified by generating polypeptides containing portions of the native antigen and evaluating the reactivity of the polypeptides with sera from *T. cruzi*-infected individuals, as described above. In many instances, peptides comprising one or more repeat sequences found in the native antigen contain an epitope. Such repeat sequences may be identified based on inspection of the above

nucleotide sequences. Representative repeat sequences for antigens encoded by the above DNA sequences are provided in SEQ ID NO:23 - SEQ ID NO:36 and SEQ ID NO:47 - SEQ ID NO:49. More specifically, repeat sequences for the sequence recited in SEQ ID NO:3 are provided in SEQ ID NO:23 (Frame 1), SEQ ID NO:24 (Frame 2) and SEQ ID NO:25 (Frame 3). Repeat sequences for the sequence recited in SEQ ID NO:4 are provided in SEQ ID NO:26 (Frame 1) and SEQ ID NO:27 (Frame 3) and repeat sequences for SEQ ID NO:9 are provided in SEQ ID NO:47 (Frame 1), SEQ ID NO:48 (Frame 2) and SEQ ID NO:49 (Frame 3). For SEQ ID NO:12, repeat sequences are provided in SEQ ID NO:28 (Frame 1), SEQ ID NO:29 (Frame 2) and SEQ ID NO:30 (Frame 3). SEQ ID NO:31 recites a repeat sequence for SEQ ID NO:15. For SEQ ID NO:16, repeat sequences are provided in SEQ ID NO:32 (Frame 2) and SEQ ID NO:33 (Frame 3). Finally, repeat sequences for SEQ ID NO:18 are provided in SEQ ID NO:34 (Frame 1), SEQ ID NO:35 (Frame 2) and SEQ ID NO:36 (Frame 3).

The polypeptides described herein may be generated using techniques well known to those of ordinary skill in the art. Polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, can be synthesized using, for example, the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* *85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc., Foster City, CA. Thus, for example, polypeptides comprising the above repeat sequences or portions thereof, may be synthesized by this method. Similarly, epitopes of other native antigens, or variants thereof, may be prepared using an automated synthesizer.

Alternatively, the polypeptides of this invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are *E. coli*, yeast, an insect cell line (such as *Spodoptera* or *Trichoplusia*) or a mammalian cell line, including (but not limited to) CHO, COS and NS-1. The DNA sequences expressed in this manner may encode naturally occurring proteins, such as full length antigens having the amino acid sequences encoded by the DNA sequences of SEQ ID NO:1 - SEQ ID NO:22, portions of naturally occurring proteins, or variants of such proteins. Representative polypeptides encoded by such DNA sequences are provided in SEQ ID NO:37 - SEQ ID NO:46, SEQ ID NO:52, and SEQ ID NO:65.

Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably, to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be

achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography.

In another aspect of this invention, methods for detecting *T. cruzi* infection in individuals and blood supplies are disclosed. In one embodiment, *T. cruzi* infection may be detected in any biological sample that contains antibodies. Preferably, the sample is blood, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood or serum sample obtained from a patient or a blood supply. Briefly, *T. cruzi* infection may be detected using any one or more of the polypeptides described above, or variants thereof, to determine the presence or absence of antibodies to the polypeptide or polypeptides in the sample, relative to a predetermined cut-off value.

There are a variety of assay formats known to those of ordinary skill in the art for using purified antigen to detect antibodies in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that binds to the antibody/peptide complex and contains a detectable reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (*e.g.*, in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptide may be bound to the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be

a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen. Nitrocellulose will bind approximately 100 μg of protein per cm^3.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

Once the polypeptide is immobilized on the support, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20 ™ (Sigma Chemical Co., St. Louis, MO). The immobilized polypeptide is then incubated with the sample, and antibody (if present in the sample) is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to permit detect the presence of *T. cruzi* antibody within a *T. cruzi*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined

by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many sources (*e.g.*, Zymed Laboratories, San Francisco, CA and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of *T. cruzi* antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. This cut-off value is preferably the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the mean is considered positive for *T. cruzi* antibodies and *T. cruzi* infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic*

Science for Clinical Medicine, p. 106-7 (Little Brown and Co., 1985). Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for *T. cruzi* infection.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antigen is immobilized on a membrane such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of *T. cruzi* antibodies in the sample. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

The assays discussed above may be performed using one or more of the polypeptides described herein. Alternatively, the sensitivity may be improved by using epitopes of one or more additional *T. cruzi* antigens in combination with the above polypeptide(s). In particular, epitopes of TcD (disclosed, for example, in U.S. Patent No. 5,304,371), PEP-2 and/or TcE (both of which are disclosed, for example, in U.S. Serial No. 08/403,379, filed March 14, 1995) may be used in conjunction with the above polypeptide(s). The PEP-2 antigenic epitope is also discussed in Peralta et al., *J. Clin. Microbiol. 32*:971-74, 1994. The sequence of TcD is provided in SEQ ID NO:50, the sequence of TcE is provided in SEQ ID NO:51. The TcD antigenic epitope preferably has the amino acid sequence Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser (SEQ ID NO:53)or the amino acid sequence Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro (SEQ ID NO:54). The TcE epitope preferably has the amino acid sequence Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala

Lys Ala Ala Thr Ala Pro Ala (SEQ ID NO: 55) or the amino acid sequence Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala (SEQ ID NO:56), and the PEP2 epitope preferably has the amino acid sequence Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala (SEQ ID NO:57).

Additional epitopes may be present within the same polypeptide (*i.e.*, in a combination polypeptide) or may be included in separate polypeptides. Preferably, the polypeptides are immobilized by adsorption on a solid support such as a well of a microtiter plate or a membrane, as described above, such that a roughly similar amount of each polypeptide contacts the support, and such that the total amount of polypeptide in contact with the support ranges from about 1 ng to about 10 µg. The remainder of the steps may generally be performed as described above.

The polypeptides described above may also be used following diagnosis using one or more of the epitopes from TcD, TcE and/or PEP2. In this embodiment, the polypeptides of the present invention are used to confirm a diagnosis of *T. cruzi* infection based on a screen with TcD, TcE and/or PEP2. Diagnosis of *T. cruzi* infection using epitopes from TcD, TcE and/or PEP2 is described in U.S. Serial No. 08/403,379, filed March 14, 1995.

In yet another aspect of this invention, methods are provided for detecting *T. cruzi* in a biological sample using monospecific antibodies (which may be polyclonal or monoclonal) to one or more epitopes, as described above. Antibodies to purified or synthesized polypeptides may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve

the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Monospecific antibodies to epitopes of one or more of the polypeptides described herein may be used to detect *T. cruzi* infection in a biological sample using any of a variety of immunoassays, which may be direct or competitive. Suitable biological samples for use in this aspect of the present invention are as described above. Briefly, in one direct assay format, a monospecific antibody may be immobilized on a solid support (as described above) and contacted with the sample to be tested. After removal of the unbound sample, a second monospecific antibody, which has been labeled with a reporter group, may be added and used to detect bound antigen. In an exemplary competitive assay, the sample may be combined with the monoclonal or polyclonal antibody, which has been labeled with a suitable reporter group. The mixture of sample and antibody may then be combined with polypeptide antigen immobilized on a suitable solid support. Antibody that has not bound to an antigen in the sample is allowed to bind to the immobilized antigen, and the remainder of the sample and antibody is removed. The level of antibody bound to the solid support is inversely related to the level of antigen in the sample. Thus, a lower level of antibody bound to the solid support indicates the presence of *T. cruzi* in the sample. To determine the presence or absence of *T. cruzi* infection, the signal detected from the

reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. Such cut-off values may generally be determined as described above. Any of the reporter groups discussed above in the context of ELISAs may be used to label the monospecific antibodies, and binding may be detected by any of a variety of techniques appropriate for the reporter group employed. Other formats for using monospecific antibodies to detect *T. cruzi* in a sample will be apparent to those of ordinary skill in the art, and the above formats is provided solely for exemplary purposes.

In another aspect of this invention, vaccines and pharmaceutical compositions are provided for the prevention of *T. cruzi* infection, and complications thereof, in a mammal. The pharmaceutical compositions generally comprise one or more polypeptides, containing one or more epitopes of *T. cruzi* proteins, and a physiologically acceptable carrier. The vaccines comprise one or more of the above polypeptides and an adjuvant, for enhancement of the immune response.

Routes and frequency of administration and polypeptide doses will vary from individual to individual and may parallel those currently being used in immunization against other protozoan infections. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 4 doses may be administered for a 2-6 week period. Preferably, two doses are administered, with the second dose 2-4 weeks later than the first. A suitable dose is an amount of polypeptide that is effective to raise antibodies in a treated mammal that are sufficient to protect the mammal from *T. cruzi* infection for a period of time. In general, the amount of polypeptide present in a dose ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the animal, but will typically range from about 0.01 mL to about 5 mL for 10-60 kg animal.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention.

Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Such adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Preparation of DNA Encoding *T. cruzi* Antigens

This Example illustrates the preparation of genomic and cDNA molecules encoding *T. cruzi* Antigens.

A. Preparation of Genomic Clones

A genomic expression library was constructed from randomly sheared *T. cruzi* genomic DNA (Tulahuen C2 strain) using the Lambda ZAP expression system (Stratagene, La Jolla, CA) according to the manufacturer's instructions. In one screen, the library was screened with a pool of sera from five patients that displayed high reactivity with parasite lysate and/or one or both of the *T. cruzi* antigens TcD and TcE, described in U.S. Patent No. 5,304,371 and U.S. Serial No. 08/403,379, filed March 14, 1995. Each of the five patients' sera was determined to be reactive based on Western and ELISA assays with whole lysate and/or TcD or TcE. Anti-*E. coli* reactivity was removed from the serum prior to screening by adsorption. 50,000 pfu of the unamplified library was screened with the serum pool and plaques expressing proteins that reacted with the serum were detected using protein A-horseradish peroxidase (with the ABTS substrate). A subsequent screen was then performed with a pool of sera from three patients lacking detectable anti-TcD antibody in Western and ELISA assays using recombinant TcD.

A similar screen was performed using a pool of sera that displayed low reactivity with lysate, TcD and TcE (*i.e.*, detected a signal less than 3 standard deviations over background reactivity in an ELISA or Western assay), followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody, as described above.

Twenty-eight clones that expressed proteins which reacted with both pools of sera in at least one of the above screens were then isolated. Excision of the pBSK(-) phagemid (Stratagene, Inc., La Jolla, CA) was carried out according to the manufacturer's protocol. Overlapping clones were generated by exonuclease III digestion and single-stranded templates were isolated after infection with VCSM 13 helper phage. The DNA was sequenced by the dideoxy chain termination method or by the Taq di-terminator system, using an Applied Biosystem automated sequencer, Model 373A.

Of the 28 clones, five had been reported previously, two were identical, and eight contained identical peptide sequences represented by a degenerate 42 base pair repeat. SEQ ID NO:16 shows the prototype clone containing the 42 base pair repeat sequence. Accordingly, 14 novel DNA sequences encoding *T. cruzi* antigens were prepared using the above screen with the reactive pool of sera (shown in SEQ ID NO:1 - SEQ ID NO:16, where SEQ ID NO:4 and SEQ ID NO:5 represent the 5' and 3' ends, respectively, of a single clone, SEQ ID NO:9 and SEQ ID NO:10 represent the 5' and 3' ends, respectively, of a single clone. One novel sequence was obtained with the screen employing the sera with low reactivity (shown in SEQ ID NO:17 (5' end) and SEQ ID NO:18 (3' end)).

B. Preparation of cDNA Clones

Poly A+ RNA was purified from the intracellular amastigote stage of *T. cruzi* (Tulahuen C2 strain). The RNA was reverse transcribed and used in the construction of a unidirectional cDNA expression library in the Lambda UniZap expression vector (Stratagene, La Jolla, CA) according to the manufacturer's instructions. 50,000 pfu of the unamplified library was screened with a serum pool containing patient sera that displayed both high and low serological reactivity, followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody, as described above. A total of 32 clones were isolated from this screen. Twenty-five of these clones were proteins of the translational apparatus that have been previously identified as highly immunogenic, and all were different from the clones identified by screening the genomic expression library. The remaining seven are represented by the sequences provided in SEQ ID NO:19 - SEQ ID NO:22. The sequence recited in SEQ ID NO:22 is that of *T. cruzi* ubiquitin.

Example 2

Synthesis of Synthetic Polypeptides

This Example illustrates the synthesis of polypeptides having sequences derived from *T. cruzi* antigens described herein.

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HBTU (O-benzotriazole-N,N,N',N'-tetramethyuronium hexafluorophosphate) activation. A gly-cys-gly sequence may be attached to the amino or carboxyl terminus of the peptide to provide a method of conjugation or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanediol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the

peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1%TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides are characterized using electrospray mass spectrometry and by amino acid analysis.

This procedure was used to synthesize peptides such as Tcc22-1,Tcc22-1+, Tcc22-2.1 (contained within SEQ ID NO:41), TcLo1.1,1.2 and 1.3 (contained within SEQ ID NOs 34, 35 and 36) and TcHi10.1 and 10.3 (SEQ ID NOs 26 and 27) which have the following sequences:

Tcc22-1 VRASNCRKKACGHCSNLRMKKK

Tcc22-1+ EALAKKYNWEKKVCRRCYARLPVRASNCRKKACGHCSNLRMKKK

Tcc22-2.1 VLRLRGGVMEPTLEALAKKYNWEKKVCRRCYARL

TcLo1.1 GYVRGRKQRWQLHACGYVRGRKQRRQLHACGYVRGRKQRWQLHAF

TcLo1.2 GTSEEGSRGGSSMPSGTSEEGSRGGSSMPA

TcLo1.3 VRPRKEAEVAAPCLRVRPRKEAEEAAPCLR

TcHi10.1 SVPGKRLRNSHGKSLRNVHGKRPKNEHGKRLRSVPNERLR

TcHi10.3 EAEELARQESEERARQEAEERAWQEAEERAQREAEERAQR

Example 3

Serological Reactivity of *T. cruzi* Recombinant Antigens

This example illustrates the diagnostic properties of several recombinant antigens found to be serologically active. This includes studies of reactivity with *T. cruzi* positive and negative sera as well as cross reactivity studies with sera from patients with other diseases.

Assays were performed in 96 well plates (Corning Easiwash, Corning, New York). Wells were coated in 50µl of carbonate coating buffer pH 9.6. For *T.cruzi* lysate, 100ng/well was used, and for each of the recombinant antigens 200ng/well was used. The wells were coated overnight at 4°C (or 2 hours at 37°C). The plate contents

were then removed and wells were blocked for 2 hours with 200µl of PBS/1%BSA. After the blocking step, the wells were washed five times with PBS/0.1% Tween 20™. 50µl of sera (either positive or negative for *T. cruzi* infection), diluted 1:50 in PBS/0.1% Tween 20/0.1%BSA was then added to each well and incubated for 30 minutes at room temperature. The plates were then washed again five times with PBS/0.1% Tween 20™.

The enzyme conjugate (horse radish peroxidase-Protein A, Zymed, San Francisco, CA) was then diluted 1:20,000 in PBS/0.1% Tween 20™/0.1%BSA, and 50µl of the diluted conjugate was added to each well and incubated for 30 minutes at room temperature. Following incubation the wells were again washed five times with PBS/0.1% Tween 20™. 100µl of the peroxidase substrate, tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added, undiluted, to each of the wells and incubated for 15 minutes. The reaction was stopped by the addition of 100µl of 1N H_2SO_4 to each well, and the plates were read at 450nm.

Figure 1 shows the reactivity of the recombinant rTcc6 (SEQ ID NO:39) as compared to that of *T. cruzi* lysate. Based on a cutoff of the mean of the negatives plus 3 standard deviations, 49 out of 50 serum samples were positive with lysate, and 34 out of 50 were positive with rTcc6. In a similar study (shown in Figure 2), the recombinant rTcc22 (SEQ ID NO:41) was found to have a sensitivity of 79.2% (38 out of 48 serum samples were positive). Comparative studies of the recombinant rTcc38 (SEQ ID NO:38) with *T. cruzi* lysate using similar criteria showed that 24/39 were positive compared with 39/39 for lysate (Figure 3). Tcc38 when tested with potentially cross reacting sera showed improved specificity over *T. cruzi* lysate.

The recombinant TcHi12 (SEQ ID NO:37) was also found to be immunoreactive (Figure 2) having a sensitivity of 62.5% (15/24).

Example 4

Serological Reactivity of *T. cruzi* Synthetic Peptide Antigens

This example illustrates the diagnostic properties of several of the peptides described in Example 2. These peptides were tested for reactivity with *T.cruzi* positive and negative sera and, in some cases, for cross reactivity with sera from patients with other, potentially cross reactive, diseases.

The first group of peptides included different reading frames to determine the most reactive repeat sequence. The peptides tested were TcLo1.1 (contained within SEQ ID NO:34), TcLo1.2 (contained within SEQ ID NO:35) and TcLo1.3 (contained within SEQ ID NO:36), representing reading frames 1, 2 and 3 of the DNA sequence provided in SEQ ID NO:18, and TcHi10.1 (SEQ ID NO:26) and

TcHi10.3 (SEQ ID NO:27) which represent two of the reading frames for the TcHi10 sequence (shown in SEQ ID NO:5). The data is shown in Figure 4. In the case of the TcLo frames, both the TcLo1.1 and 1.2 peptides were strongly reactive but the TcLo1.2 was superior in signal to noise when tested on sera from *T. cruzi* positive and negative individuals. TcLo1.3 had lower signal but also low background. In this study lysate detected 24/24 positives, TcLo1.1 detected 21/24, TcLo1.2 detected 23/24 and TcLo1.3 detected 15/24. In the same study, the two frames TcHi10.1 and 10.3 detected 19/24 and 14/24 positives respectively, but with lower signal than for TcLo1. Cross reactivity studies with these different reading frames demonstrate that TcLo1.2 has minimal cross reactivity with the sera tested (Figure 5) as compared to *T.cruzi* lysate.

As discussed in Example 2, overlapping peptides were also synthesized for rTcc22 to determine the active epitope. The peptides Tcc22-1, 1+ and 2 were tested with *T. cruzi* positive and negative sera. The results are shown in Figure 2. The Tcc22-1+ and Tcc22-2.1 peptides were more reactive than the Tcc22-1 peptide. In the first experiment, Tcc22-1 and Tcc22-1+ detected 29/48 and 36/48 positives as compared to the recombinant Tcc22 which detected 38/48 positives. In a subsequent experiment, Tcc22-2.1 was also shown to be reactive but with less signal than Tcc22-1+ at the same plate coating level.

A polypeptide having the TcHi15 frame 3 repeat sequence (SEQ ID NO:49) was also synthesized and tested in an ELISA assay using a coating level of 200 ng/well. A total of 48 *T. cruzi* positive sera and 26 negative sera were tested in order to determine the reactivity of this peptide sequence. In this study, the peptide had a sensitivity of 68.75% (detecting 33 out of 48 positives) and a specificity of 92.3% (24 out of 36 negatives), indicating that this polypeptide has potential significance in detecting *T. cruzi* infections. The results of this assay are presented in Table 1, below.

Table 1

Reactivity of TcHi15 Frame 3 Polypeptide with *T. cruzi*-Positive and Negative Sera

Sample ID	*T. cruzi* Status	OD 450	Sample ID	*T. cruzi* Status	OD 450
Tc011095-1	Positive	0.696	DL4-0106	Negative	0.167
Tc011095-2	Positive	0.699	DL4-0112	Negative	0.05
Tc011095-3	Positive	1.991	DL4-0127	Negative	0.240
Tc011095-4	Positive	3	DL4-0140	Negative	0.008
Tc011095-5	Positive	0.098	DL4-0145	Negative	0.107
Tc011095-6	Positive	0.238	DL4-0161	Negative	0.119
Tc011095-7	Positive	0.115	DL4-0162	Negative	1.187
Tc011095-8	Positive	0.156	DL4-0166	Negative	0.210
Tc011095-9	Positive	0.757	DL4-0167	Negative	0.131
Tc011095-10	Positive	1.147	DL4-0172	Negative	0.073

Sample ID	*T. cruzi* Status	OD 450	Sample ID	*T. cruzi* Status	OD 450
Tc011095-11	Positive	0.264	DL4-0175	Negative	0.117
Tc011095-12	Positive	1.7	DL4-0176	Negative	0.815
Tc011095-13	Positive	1.293	AT4-0013	Negative	0.100
Tc011095-14	Positive	0.242	AT4-0041	Negative	0.107
Tc011095-15	Positive	0.636	AT4-0062	Negative	0.28
Tc011095-16	Positive	0.44	AT4-0063	Negative	0.155
Tc011095-17	Positive	3	E4-0051	Negative	0.162
Tc011095-18	Positive	1.651	E4-0059	Negative	0.176
Tc011095-19	Positive	0.19	E4-0068	Negative	0.241
Tc011095-20	Positive	0.916	E4-0071	Negative	0.127
Tc011095-21	Positive	0.715	C4-0072	Negative	0.101
Tc011095-22	Positive	1.336	C4-0088	Negative	0.141
Tc011095-23	Positive	1.037	C4-0090	Negative	0.078
Tc011095-24	Positive	0.332	C4-0096	Negative	0.162
Tc011095-25	Positive	0.413	C4-0101	Negative	0.181
Tc011095-26	Positive	0.266	C4-0105	Negative	0.702
Tc011095-27	Positive	1.808			
Tc011095-28	Positive	0.238			
Tc011095-29	Positive	0.266			
Tc011095-30	Positive	1.563			
Tc011095-31	Positive	0.352	Sensitivity	33/48	68.75%
Tc011095-32	Positive	0.208	Specificity	24/26	92.30%
Tc011095-33	Positive	0.656	Mean Pos.	0.9188	
Tc011095-34	Positive	1.281	Std Dev Pos.	0.79	
Tc011095-35	Positive	0.907	Mean Neg.	0.1508	
Tc011095-36	Positive	0.429	Std Dev Neg.	0.06695	
Tc011095-37	Positive	0.454			
Tc011095-38	Positive	0.725			
Tc011095-39	Positive	0.703			
Tc0394-7	Positive	0.186			
Tc0394-8	Positive	1.06			
Tc0394-9	Positive	1.813			
Tc0394-10	Positive	0.131			
Tc0394-11	Positive	1.631			
Tc0394-12	Positive	0.613			
Tc0394-13	Positive	3			
Tc0394-14	Positive	0.268			
Tc0394-15	Positive	2.211			

Example 5

Serological Reactivity of Peptide Combinations

This example illustrates the diagnostic properties of several peptide combinations.

The TcLo1.2 peptide (contained within SEQ ID NO:35) was tested in combination with the synthetic peptide TcD and also the dual epitope peptides D/2 (which contains the TcD and the PEP-2 sequences) and D/E (which contains TcD and

TcE sequences). These combinations were compared with the individual peptides as well as the tripeptide 2/D/E, which contains TcD, TcE and PEP-2. The TcD sequence used was Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser (SEQ ID NO:53), the TcE sequence was Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala (SEQ ID NO: 55), and the PEP2 sequence was Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala (SEQ ID NO: 57).

The data are shown in Figure 6. The results show that TcLo1.2 can augment the reactivity of TcD, D/2 and D/E, as summarized in Table 2.

Table 2

Sensitivity of Peptide Combinations in the Detection of *T. cruzi* Infection

Peptides	Number of Positives
TcD	62/67
TcE	50/67
PEP-2	66/67
TcLo1.2	61/67
TcD+TcLo1.2	66/67
D/2+TcLo1.2	67/67
D/E+TcLo1.2	67/67
2/D/E	67/67

These results demonstrate the use of *T. cruzi* antigens as described herein to enhance the serodiagnostic properties of other antigens.

Example 6

Serological Reactivity of TcE Repeat Sequences

This example illustrates the diagnostic properties of several TcE repeat sequences.

The repeat sequence region of the recombinant TcE contains several degeneracies, resulting in residues where an A (alanine), T (threonine) or I (isoleucine) can be present in the repeat sequence. In order to represent all degeneracies, the original sequence for the synthetic TcE peptide was made with an A, T and I in a single peptide containing three repeats (*see* Example 5). In order to further epitope map the

repeat region and to determine the number of repeats required for serological activity, the following peptides were prepared as described in Example 2:

original TcE	KAAIAPAKAAAAPAKAATAPA (SEQ ID NO:55)
TcE(3A)	KAAAAPAKAAAAPAKAAAAPA (SEQ ID NO:58)
TcE(3T)	KAATAPAKAATAPAKAATAPA (SEQ ID NO:59)
TcE(3I)	KAAIAPAKAAIAPAKAAIAPA (SEQ ID NO:60)
TcE(2A)	KAAAAPAKAAAAPA (SEQ ID NO:61)
TcE(AT)	KAAAAPAKAATAPA (SEQ ID NO:62)

The serological reactivity of these peptides was then compared. A total of 24 positive and 21 negative sera were tested with each of the TcE variants as the solid phase in an ELISA assay performed as described in Example 3, using 25 ng/well of peptide. The reactivity of the different peptides is shown in Figure 7. The highest reactivity was seen with the 3-repeat peptide in which each repeat contained an A at the degenerate residue (TcE(3A)). This peptide displayed even higher reactivity than the original TcE sequence containing an A, T and I residue in the three repeats. The 3I and 3T variants by contrast were essentially negative with the *T. cruzi* positive samples tested. The sequence containing two repeats with A (TcE(2A)) was clearly less reactive than the 3A sequence and the two repeat sequence with an A and a T (TcE(AT)) was negative. Based on a cutoff of the mean of the negatives plus three standard deviations, the original TcE (A,T,I) detected 17 out of 24 positives and the 3A variant detected 19 out of 24 positives. It also appears that to obtain maximal serological activity at least three repeats are required.

Example 7

Serological Reactivity of Multi-epitope Peptide Combinations

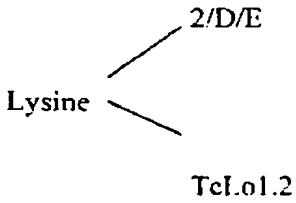
This example illustrates the diagnostic properties of several multi-epitope peptide combinations.

Two dipeptides PEP-2/TcLo1.2, which contains the PEP-2 (SEQ ID NO:57) and TcLo1.2 (SEQ ID NO:35) sequences, and TcD/TcE, which contains the TcD (SEQ ID NO:53) and TcE (SEQ ID NO:55) sequences, were synthesized as described above in Example 2. The reactivity of these two dipeptides with *T. cruzi* antibody-positive sera was compared to that of the tripeptide 2/D/E. ELISA's were performed as described in Example 3 using PEP-2/TcLo1.2 at 250ng/well and TcD/TcE at 50ng/well. The results of this study are shown in Figure 8. One *T. cruzi* positive serum found not to react with the tripeptide 2/D/E was used in screening for the

TcLo1.2 epitope. This serum was detected by the TcLo1.2 epitope and also by the dipeptide mix (PEP-2/TcLo1.2 together with TcD/TcE) as expected.

A tetrapeptide containing the four immunoreactive *T. cruzi* epitopes PEP-2, TcD, TcE and TcLo1.2 in a linear sequence, herein after referred to as 2/Lo/2E/D (SEQ ID NO:63) was synthesized as described in Example 2. This tetrapeptide was coated at 100ng/well and its reactivity with T. *cruzi* positive and negative sera was assayed as described in Example 3. The reactivity of the tetrapeptide 2/Lo/2E/D is shown in Figure 8. The one *T. cruzi* positive serum found not to react with the tripeptide 2/D/E was detected by the tetrapeptide as expected.

The four immunoreactive *T. cruzi* epitopes PEP-2, TcD, TcE and TcLo1.2 may also be linked into one reagent by the use of a 'branched' peptide originating from a lysine core residue. Orthogonal protection of the lysine, for example employing 9-Fluorenylmethoxycarbonyl (Fmoc) on the α-amino group and 1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) on the ε-amino group, is used to permit selective deprotection of one amino group in the presence of the other, thereby allowing the synthesis of the first peptide chain from either the α- or ε- group on the lysine. This first peptide chain is terminated with a protecting group that is not removed during the course of the synthesis of the second peptide chain. For example, a tert-Butoxy carbonyl (Boc) amino acid could be used with the Dde and Fmoc combination. The remaining lysine amino protecting group is then removed before a second amino acid chain is synthesized from the second amino moiety. For example, ε-Dde is removed with 20% hydrazine. Cleavage of the branched peptide from a solid support and removal of the N-α-Boc moiety is carried out using trifluoroacetic acid, following standard protocols. Using this approach two independent amino acid sequences can be built from a 'core' lysine residue, as shown below, thus allowing various combinations of TcD, TcE, PEP2, TcLo1.2, and other epitopes to be coupled to the core residue. Purification of the resulting peptide is performed as described in Example 2.



Example 8

Comparison of the Serological Reactivity of TcHi29 and TcE

The antigen TcHi29 (SEQ ID NO:52) was shown to be a polymorph of the TcE repeat sequence. A TcHi29 peptide was synthesized that had the following sequence as compared to TcE.

TcE KAAIAPAKAAAAPAKAATAPA (SEQ ID NO: 55)

TcHi29 KTAAPPAKTAAPPAKTAAPPA (SEQ ID NO: 64)

Figure 9 shows a comparison of the reactivity of these two related seqences with sera from *T. cruzi* positive patients as well as from other disease categories, as determined by ELISA using the procedure described above. The data indicate little or no cross reactivity with the other disease groups tested but the distribution of reactivity amongst the *T. cruzi* positive sera partially overlapped for the two peptides. Of the 53 consensus positive samples tested, TcE detected 31/53 and TcHi 29 36/53. Within this group TcE and TcHi29 both detected 24 of the same sera. TcE detected 7 positive sera not detected by TcHi29, which in turn detected 12 positive sera missed by TcE. A dipeptide, TcD/TcHi29, was also synthesized and used in combination with the PEP-2/TcLo1.2 dipeptide in ELISA (100ng/well TcD/TcHi29, 250ng/well PEP-2/TcLo1.2) and compared with the TcD/TcE plus PEP-2/TcLo1.2 dipeptide combination. As shown in Figure 10, the data indicates that the overall activity of the two mixes are similar for both the *T. cruzi* positive and negative populations studied and suggests that, in such peptide combinations, TcHi29 can be considered to be an alternative to TcE.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Corixa Corporation

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR THE DETECTION AND PREVENTION OF *T. CRUZI* INFECTION

(iii) NUMBER OF SEQUENCES: 65

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SEED and BERRY LLP
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 14-NOV-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Maki, David J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 210121.422PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 622-4900
(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGGAAAAGA AGGCTGTTAC GACGCACGAG CTTGGCTTTG AGGGCGAGGA CTGGGACTAC 60

GTGCTGGAGC GGCGGCGCGC GGAGGTGAAG GACGTGCTGG CCGTCGAGAC GGCGCGGGCG 120

TTGGGACTCG AGCGTGAGGA CGTGCTGGAG GTGGAGGTCG ACGCAGTGCC TCGGAGCCTC 180

ATTGCGTTTG TCACGGTCCG TCATCCATCA CTGCTGAGCG ACCGCAGGTG GAAGAGACGC 240

TGGCGCGCTG CGAGTACAGG AAATTGTGGG CGCTGTACGA GACGCGGCCA CTGGAGTCGT 300

CAGTGCTGAT GAGGCGGTTT GAGGGCGACG ACTGGGACCT CGTGGTTGAC AACAACCGCA 360

GGAAGCTTGA GGACGCGTTC AGCAGGGAGA CGGCCGCGCA CTGGGCGTGT CGCCGAGGCA 420

GGTTGTGCTT CTGGACTGCA GGGTTGGCAG CCTTCTCATG GTATTCAAGG TGCTTGGATG 480

CGCCATGAGC GACGCAGAGA TCACGGAACG GACCGAGG 518

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 560 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGGCGGTAGT CTGCGATGCT GTGGACCGAC GCATTGAAAT ACACACCGTC TTCGGCGTTC 60

CTTTTTTTTA TATGTTTTTT TTTATTGAGA AGATGTCTTG TTTGTTGTTG TTTTTTTTCA 120

GTTTTTATGA TACGAGCAGT TTGTCCGACT GCATTCATGC AGTGATTGGT AATTCTTTCT 180

ATTCTTTGGA ATTATGGCGA TATTATTCTT GTCTTTTAAA ATTCTTACAA CCAATTGTGC 240

CTTAGAGTTT CCTGCTTAGT TGCTATTAAC ACACTGTTAG GAACGCGAAA CCATGCAGAT 300

CTTCGTGAAG ACACTGACGG GCAAGACGAT CGCGCTCGAG GTGGAGTCCA GCGACACCAT 360

TGAGAACGTG AAGGCGAAGA TCCAGGACAA GGAGGGTATC CGCCGGACCA GCAGCGCCTG 420

ATCTTCGCTG GCAAGCAGCT GGAGGACGGC CGCACGCTCG CAGACTACAA CATCCAGAAG 480

GAGTCCACGC TGCACCTTGT GCTGCGCCTG CGCGGCGGCA TGCAGATCTT CGTGAAGACA 540

CTGACGGGTA AAGACGATCG 560

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGGCTGCCTC CTCTGCTTCC TTCCTCGGAC GTGCCCGAAG GCATGGAGCT GCCTCCTCTG 60

CTTCCTTCCT CGGACATACC CGAAGGCATG GAGCTGCCAC CTCTGCTTCC TTCCTCGGAC 120

GTACCCGCGG GCATGGAGCT GACACCTCTG CTTCCTTCCT CGGACGTGCC CGAAGGCATG 180

GAGCTGCCAC CTCTGCTTCC TTCCTCGGAC GTACCCGCGG GCATGGAGCT GCCACCTCTG 240

STTCCTTCCT CGGACGTACC CGCGGGCATG GAGCTGCCTC CTCTGCTTCC TTCCTCGGAC 300

GTACCCGCGG RCATAGAGCT GCCACCTCTG ATTTCCTNCC TCGGACGTAC CCNCAGGNAT 360

GGAGATGNCT CCTCTGNTTC CTGCCTCGGA CGTNCCCNAA GGNATAGAGN TGCNCCTCTG 420

NTTCCTNCCT CGGAAG 436

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 373 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTCAGGGGC TCTTGGCGTT CCTTTTTTTC TTGTTGTTTT GAGTTTTTTT TTCTTTTGTT 60

TTGGTTTGTC GTCTCTGTTT TTATGTGCGT TGTTTTCGGT TTTTCTTTTT GTTCTTCCTG 120

CCTGTCATGT GACTAGTTTT ATGTTTTCCA GGCCGACCGT CACTCAATTT TTTTATTTTT 180

ATTTTTATTT ATTTATTTGA CCCGCCTTTC TCTGTAGTTT ACGAGAGTTT AGATTTTTAT 240

TGATTGGTAG TTTAGGGCCA TCAGGCGGGA GGGGCGAGTC TGGCGGAAGA CAAAACAAAA 300

TACGATGGAC TCGACCAACA GCATCGAGAA AICGCTTCTG ATGGAGATGG AGCGGGAGGT 360

TGAGAGGGCG AGG 373

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGAAAAAGA ACGTAGATTT CCAACCAAAA CAGCAAGAGC GGATCCAACA ACGACCAAAC 60

AACTCATTAT TCGAGCTCTC CAAAATATAT CGCTTGCCTT CGGGATTGAA CCCTCATCTA 120

CAGTAAAATA CGCCGAAAGC ACGCAAGAAG AAAATGGAAA ACGTTCACAA AGTGACGCCG 180

AGGAGCGTGC ACGGCGGGAG GCTGAGGAAC GAGCACGGCG AGAGGCTGAG GAACGAGCCC 240

AACGAGAGGC TGAGGAACGA GCCCAACGAG AGGCTGAGGA ACGAGCACGG CGGGAGGCTG 300

ACAAGCGTGC CCGGCGAGAG GCTAAGGAAC GAGCATGGCA AGAGGCCGAA GAACGAGCCC 360

AACGAGAGGC TGAGGAGCGT GCCCGGCGAG AGGCTGAGGA GCGTGCCCGG CGAGAGGTTG 420

AGGAGCGTGC CCGGCAAGAG GCTGAGGAAC TCGCACGGCA AGAGTCTGAG GAACGTGCAC 480

GGCAAGAGGC CGAAGAACGA GCATGGCAAG AGGCTGAGGA GCGTGCCCAA CGAGAGGCTG 540

AGGAGCGTGC TCAACGAGCG 560

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 440 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCCTCCTGCA ACTCGAGCTG GCAGCGTGGA GGTGCNGCAG GAACTCTCAA NAGANGACGC 60

CTCTCCCTCG ATANCNTTCG GAGTGACTTN GACTGTTGCG CCNTTTCCGT NTCACTATTT 120

CTATTGCTTT TAATTTGCTG GAGAGGCGCG TGTAGGAGGG AAAGAGTAGT AACATGGCAG 180

AATCATCAAA AACGATGTTG CGTTAGTAGA GAGGAGGGAA ACATCGAGAC GTTGAGGGTT 240

GCGACGGNCA AAATTATGTA CATTTACCTG AATTAGGATA AGACTTCATA TGGCATAAAC 300

TCGTGGCGTT GTTGGTGGTT ATAACAAGCA ACGGTGACGA TGTCTTAGGC TACACTGCTG 360

CACTCAAAGA GTTTTACAGG TACTTGCGGG ATATTTGTTC CTGTGAGTTT GTTTTCTATT 420

GTAATTTATT NNGTCTCAAT 440

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1915 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGATGCGTCT GTCGTAGACC TGGGAGGCGA GGCCCATGGG ACACACTATG CCTTTTTGCC 60

CGATGTGATC AAGGGGATTG CGCAGGAAGA GCTGTACCTG GAAGACGATG CGTACTTCCA 120

GGAGTTGCTT GCGAGGTATA AAGAACTTGT CCCTGTGGGT GCCGAGCCAA CCGAGCCACC 180

CGCAAAGCAG TTGCGCGAGC AAATGCGGAT ACGGGCTGGG CAGCTTGCTG TTGACACCCG 240

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TGAGTTGGCT CTCATGTCTG ATCCCAATTT TGCGGAGCTG GCGACACGGC ACGCGCAGGA 600

GGCGACCTCG GGCGATGCGG CGGGTATTTT GCGCCTTGAG CAGGAGCTGC GTGACCAGGC 660

ATGTCGCATA GCACGTGAGG TGCGAGTGGC TCGGCGGCTT GACGCCGTCG CAATGAGGAC 720

CTGCACGAGC GGTACCCGTT TCTTCCCGAG GAGCCGGTGC GCGGCATTCT TCTTGGTGCT 780

GTGCGTCCGG TGCAGCAACC GGCGTTCCGC GAGCTTTCAA ACAAGTTGGA TGAGCAGCGC 840

CGGGACCCGA CACGCAACGC AGCCGCGATC CGCACGACGG AGGAGCAGAT GACTGCGTTG 900

GTGGTGCGAC TGGCTGAGGA GCGCGCGGAG GCGACGGAGA GGGCGCATGA GCAGTACCCG 960

TTTCTCCCAC GACGTGTGCT GGGCGTGCGC CTTGGTGACA TCTCGCTGCA GGAGGATGAT 1020

GTGTTGTCAC AGCTGGCGCG GCGTCGTGTG CGGCAGCTAA GAAACTCCAA GACGGCGATT 1080

GACGCACACG CAACTGAAGA AGAGATGATA AGGCGCGCAG AGGAGCTGGC TCGCAACGTG 1140

AAGCTTGTCG ACGCATACCG TGGGAATGGG AACGAGTACG TGCGTGCCTG CAACCCGTTT 1200

CTCGTGTACG AGGACCGCAA GTGCGTCCTC CTGAGTGAGC TGCCGCTTGC CGGTGGCGAC 1260

GTGTACCAGG GCTTGTTCCG GGATTATCTG ACTGCGCTGG AGGACGCCGA GGCAAATGCA 1320

CCGCGGATCG CGGAGCTGGA GAATGCGCTT CGGTCCCGTG CGGATGAGTT GGCGCTGGAG 1380

GTTTGCGAGA GGGACGCGCG GTTGTTGCAT TACTCATTCC TCTCGGCCCA GGATGTTCCT 1440

GGTTGGTCTG AAGCACTGCT GCATGACGCG GAGTTTCAGC AGCTACGTGA GCGTTACGAG 1500

GAACTGAGCA AGGATCCACA GGGGAACGCC GAGGCATTGC GTGAGCTTGA GGATGCAATG 1560

GAGGCTCGGA GCAGAGCCAT TGCGGAAGCG TTGCGGACTG CAGAGCGACT AATCCACTGA 1620

GCAGGCGAGG CTGAAGACGC CGTCACAGGC GGGGTCTGGC GTGTCCGCGG GTGATCGAAT 1680

GCATGGCAGC GAGCATGCGG ATCTCGCGCA TGAAGGGGGA AGCACGGCTG GCGGCACCAT 1740

GAGGGGGGCA GAGTCTGTCT CCAAGAGCAG TGGGAAACAC TCTCAAGGTC GGTCTCGCAT 1800

GCGTCTGTCG TAGACCTGGG AGGCGAGGCC CATGGGACAC ACTATGCCTT TTTGCCCGAT 1860

GTGATCAAGG GGATTGCGCA GGAAGAGCTG TACCTGGAAG ACGATGCGTA CTTCG 1915

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTACCAAGCT GAGATAGATA AAAGGCTGCA GGAGCAGCTT GCCCCTGAGA GGATGAGGGC 60

TCTTTCCGCA TTTCTTTCGG AGTGACTTTG ACTGTTGCGC CGTTTCCGTG TCACTATTTC 120

TATTGCTTTT AATTTGCTGG AGAGGCGCGT GTAGGAGGGA AAGAGTAGTA ACATGGCAGA 180

ATCATCAAAA ACGATGTTGC GTTAGTAGAG AGGAGGGAAA CATCGAGACG TTGAGGGTTG 240

CGACGGNCAA AATTATGTAC ATTTACCTGA ATTAGGATAA GACTTCATAT GGCATAAACT 300

CGTGGCGTTG TTGGTGGTTA TAACAAGCAA CGGTGACGAT GTCTTAGGCT ACACTGCTGC 360

ACTCAAAGAG TTTTACAGGT ACTTGCGGAT ATTTGTTCCT 400

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 936 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCCTCCTGCA ACTCGTGCTG GCAGCGTTGA AGTTCGGCAG AAATCTCAAC AAACGCCTTC 60

TGTCCCTCGG AAACCTTCCC GTTAAGAGAC ACAAGCAGTT CAATGAGCGA CATGGTCGCT 120

TCGGACACGT CCAATGCTTT CATGGTTTGT TCCAGCCGCC GCTGAAAGTT ATCCACACAT 180

GAGAACAACA AAGACAAATC TAAATCGGCG TCGCCGTGCT CATACACATC AAACGCCACC 240

GTCTCGCCCA AAACATTCAA AAAGTTCACC AAAAAGTTTA CAAGCTTACT CAAATTGTCA 300

CGAAGTGAGC TAACGGTAAT TTCTAAACTT CCATTTCTTG CGTCATCCCT AGCCTTCGCC 360

GCGACTACCT TCTCCTTCCA TAGCACTAGC TTCTCCTCCA CCAAACGAAT ACCGCTCTCC 420

TTTTCTTTCA CAGCAACCTC ACATTCCCTT TCAATTTCAT TCAACCTAAT TGGATTATTT 480

TCTTAAACGA CTTGCCGTGC CCTCCTCGGG CTGATGAAAG GCCTCGCCCA GCTGCGCACC 540

CAGATTCACG GTGTCCGCCC CGTTCTGCTC CCGGAGAGCG GCCAGTTCCT CGGTGGTTCG 600

CTTCAGCTCG CGATGCACCT CCTCGCGCTG CTGCAAGGCC TCGTCCAGCT GCCCACGCAG 660

ATTCACGGTG TCCGCCCCGC TCTGCTCCCC GAGAGCGGGC AGTTCCTCGG TGGTTCGCTT 720

CAGCTCGCGA TGCACCTCCT CGCGCTGCTG CAAGGCCTCG TCCAGCTGCG CACGCAGATT 780

CACGGTGTCC GCCCCGCTCT GCTCCCGGAG AGCGGGCAGT TCCTCGGTGG TTCGCTTCAG 840

CTCGCGACGC ACCTCCTCGC GCTGCTGGAA GGCCTCGCCC AGCTGCGCAC GCAGATTCAC 900

GGTGTCCGCC CCTCTCTGCT CCCGGAGGGC GGGCAG 936

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 702 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACTTGAAAGA NTGACCCAAT AATNGGGTTC CTTATTGTGC CACCCCAAAT AAACCCGTAA 60

CCAATTTGTG GCTGGGATGG ATCCCCCCAC NCTCTTTGAC NCATGTCAAG AGTANATGGG 120

ACGTCAAAGT CACTTAGAGA GGGATTCATG GGTNCCATTG ATCACAAGAG CCTNCTGGAA 180

CACCCCCGTG AAGATAACCC AATGAGATTT ATCGTCTGCA TAAGATCACA CGAGGCGGTA 240

TTAGCAATTA TCTTCACAGA TTCTTTTTCT TGTGATGGTG GCTTGCGGTA GTTTGTCATC 300

ATTGTTTTCT GAATGCAATG AAGCACACGA CTTGTAATAC GTTCTCCATG TCTTTCAATC 360

GTTTCCAACG CCTCCACAAT GTCTGCAGGA TCCCCAGGAA GGTCAGCAGT CATCAGAAGC 420

TCTTCACATG AACGCCGTAA ACTAGGATCA CGCTCAACAA GGCTAGCAAT CGCATTTGCC 480

ATTCTCGGAT TCCACTTGCA AAACCACTCC GGAAGTTTAT TTCCACGACT GACCTCTGTC 540

ATAATGTTGA ACCTCTCCCT AAAGCCTTTA CCCGCCACGG CAAGCCACAT CTCAAGAGCT 600

ATCATACCCA GGCTGTATTC ATCCACTTTA AAGTCGTAGT CTTCCCCTCG CTCTTGCTCT 660

GGGGCACAGT ACAACACAGA ACCCAAGTTT CCTGTAGGAC CG 702

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 510 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGAGTATTCC TGTGGAAATT GATATTAGAA ACCAGGACTT TTCTTTTCTT GACCCGGCAC 60

CGGAGGGCAT TCCTATTCAG GACATACATC TTATGGGAGA TTCTGCATTT GCCGCATCTG 120

CGCGTGAGCG CATGAAACTG AAAAGAAATC CTGTTGCGAA TGCGAGCAAG ATCAGTGCCC 180

TTGAGGAGGA GATGGATCAA CGTGCTCATG TATTGGCTAA GCAGGTGCGT GACAAAGAGC 240

GCACTTTCCT TGATCCAGAG CCTGAGGGTG TTCCACTTGA GTTGCTTTCA TTAAATGAAA 300

ATGAGGCCTC ACAGGAATTG GAGCGAGAGC TTCGTGCCCT AAATCGCAAA CCCCGGAAGG 360

ATGCCAAAGC AATAGTTGCT CTTGAAGATG ATGTGCGTGA CGAACACACG TGCTTGCCAA 420

GGAGCTAAAG GAAAATGAGC GGAACATCTT TGTTGGCTCC ACAGCCTGAG GGTGTGCCGG 480

TGTCTGAGCT GTCGTTGGAT TTAGACGAGC 510

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 320 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGGTCGTGGC AGAGCCAAAG CCACCAACAG CAGGTGCCGA CGTGTGCGCG GCAGAGCCGA 60

AGCCACCAGC AGCAGGCGCC GAAGTGGTCG TGGCAGAGCC AAAGCCACCA GCAGCAGGTG 120

CCGACGTGTG CGCGGCAGAG TCGAAGCCAC CAACAGCAGG TGCCGACGTG GTCGTGGCAG 180

AGCCAAAGTC ACCAGTAGTA GGNGCCGACG TGTGNGTGGC AGAGNCANAG NCACCAGTAG 240

NAGGTGNCGA CGTNGTCGTC GNAGAGNCGA NGTCACCAGC AGGAGGTGNC GACGTNTGNG 300

NGGNAGAGGC GATGTCACCA 320

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 302 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGCATCTCC CCCGTACATT ATTTTGCGGA AAATTGGATT TTTACGGGGA GGTGGGGTTC 60

GATTGGGGTT GGTGTAATAT AGGTGGAGAT GGAGTGCAGT GGGATAGGAT TAGAATGTAG 120

TTGGTGTAGT ACAGAGTTTA TATAGTATAG TGTTGATGTT ATTATACAAT GAGGTAAGAG 180

AATGGAGTGA GAAAGAGTAT GTTTGTTAGT TTGGTTGTTA ATGTTATGTA TTCATGTTAT 240

CAGTATATGT TGTATGTGTA TGGTGATAGC GGTGGGTGTA GCTGTATGTG GTAGGTTAGA 300

GT 302

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 298 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAGTTTCAAT TTCCTCTCCA CCTGATCCCG CTGTTGCAAA AGCGTCCTTG ATGTATCCTG 60

CTCCTTTGCC GCTAGCGCCT CCCTTGCTAA GCGCAGTTCC TCTTGCAGCC TCGCCTGCAC 120

CCGTTCCGCC TCCATTAATC TCTTCTCCCC GATTGCTTCT TTGGCGCGTA AATCCTCCAG 180

TTCCTTCTCT ATCAAAGTGT GCCTCCCATT CCTGATCCGC GACTCTTCAC AGGCTTCTTG 240

CTCCGCGTCA CGGAGACGCC TCTTGAGAGC CTCGTCTTC TCTTCCAGGT CTTCTGGG 298

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2144 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGAATTC TTACCAAGCT GAGATAGATA AAAGGCTGCA GGAGCAGCTT GCCCCTGAGA 60

GGATGAAGGC TCTTTCCACA TTTCTTTGGG AGTGACCTTG ACTGTTGCGC CGTTTCCGTG 120

TCACTATTTC TATTGCTTTT AAATTGCTGG AGAGGCGCGT GTAGGAGAGA AAGAGTAGTA 180

ACATGGCGGA ATCATCAAAA ACGATGTTGC GTAAGTAGAG AGGAGGGAAA CATCGAGACG 240

TTGAGGGTTG CGACGGCCAA GATTATGTAC ATTTACCTGA ATTAGGATAA GACTTCATAT 300

GGTATAAAGT CGTGGCGTTG TTGGTGGTTA TAACAAGCAA CGGTGACGAT GTCTCAGTCT 360

ACACTGCTAC AATCAAAGAG TTTTACAGGT ACTTGTGGAT ATTTGTTCCT GTGAGTTTGT 420

TTTCTATTAT AATTTATTTT GTCTCAATTT TTTGTTTCCC CGCTTCCTAC GGTCTCTTTT 480

TTTCTTCGTT CTTGAAATTT CAATTATTGC TTAACCACAA GCATCCAGTA CTTCAACCTC 540

CCCATCAAAT GGTGTCGCTG AAGCTGCAGG CTCGTTTGGC GGCGGACATT CTCCGCTGCG 600

GTCGCCACCG TGTGTGGCTG GACCCTAATG AGGCCTCTGA GATTTCCAAT GCAAACTCGC 660

GCAAGAGCGT GCGCAAGTTG ATCAAGGATG GTCTGATTAT TCGCAAGCCT GTCAAGGTGC 720

ACTCGCGCTC CCGCTGGCGC CACATGAAGG AGGCGAAGAC CATGGGCCGC CACGAGGGCG 780

CTGGGCGCCG CGAGGGTACC CGCGAAGCCC GCATGCCGAG CAAGGAGCTG TGGATGCGCC 840

GTCTGCGCAT TCTCCGCCGC CTGCTGCGCA AGTACCGCGA GGAGAAGAAG ATTGACCGCC 900

ACATTTACCG CGAGCTGTAC GTGAAGGCGA AGGGGAACGT GTTTCGCAAC AAGCGTAACC 960

TCATGGAGCA CATCCACAAG GTGAAGAACG AGAAGAAGAA GGAAAGGCAG CTGCCTGAGC 1020

AGCTCGCGGC GAAGCGCCTG AAGGATGAGC AGCACCGTCA CAAGGCCCGC AAGCAGGAGC 1080

TGCGTAAGCG CGAGAAGGAC CGCGAGCGTG CGCGTCGCGA AGATGCTGCC GCTGCCGCCG 1140

CCGCGAAGCA GAAAGCTGCT GCGAAGAAGG CCGCTGCTCC CTCTGGCAAG AAGTCCGCGA 1200

AGGCTGCTGC ACCCGCGAAG GCTGCTGCTG CACCCGCGAA GGCCGCTGCT CCACCCGCGA 1260

AGACCGCTGC TGCACCCGCG AAGGCTGCTG CACCTGCCAA GGCTGCTGCT CCACCCGCGA 1320

AGGCTGCTGC TCCACCCGCG AAGACCGCTG CTCCACCCGC GAAGACCGCT GCTCCACCCG 1380

CGAAGGCTGC TGCTCCACCC GCGAAGGCCG CTGCTCCACC CGCGAAGGCC GCTGCTCCAC 1440

CCGCGAAGGC CGCTGCTGCA CCCGCGAAGG CCGCTGCTGC ACCCGCGAAG GCTGCTGCTC 1500

CACCCGCGAA GGCCGCTGCT CCACCCGCGA AGGCTGCTGC TCCACCCGCG AAGGCTGCTG 1560

CTCCACCCGC GAAGGCTGCT GCTGCTCCCG TTGGAAAGAA GGCTGGTGGC AAGAAGTGAA 1620

GCGCGCACTA GTACGACCAA CTTGTTTTTT TTTTTGGTAT TTAATATTTT CTGAGGAAGA 1680

AGTGGGTATT GAGGGTCTTT CTTTCCGCGT TTGTGTTGGT TTGTGGTGTT CGTGACATTA 1740

TAGTAGATCC AAAGTATTCT TCAGTGTCCC TTTTCCTTTT CTCCATCCTT TTTCCTATTT 1800

TTTGTTTGTC TTCTCTACGA TCTTTGTTGT CGTGTGACCT CCGCTGTATG GAACTGACGG 1860

CCGGCGTTGT GAGAGACGAT GTCGCACGTC ACGGCGGACC TGGAGTATTT TAAATGTGAC 1920

ATGTGCGGGG TGTATCTGCA CAAAGACATC TTTTGCGACC ATCGACGTGA GTGTAAAGGC 1980

CTTGATTCGA AAGAGCTGAA GAAGAGCCAG TGTCGTCAGA TCGGGATGGC ATTAGACAAG 2040

GAGGCACGGC ACCGAATTGC GTCACGAATG GCTGATGGAG CAACTCTCGT GCCTGTCGAG 2100

CTTGCAGAAC GACATCAACA GGCGCGTGTG CGGCGTAATG TGGC 2144

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 456 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGTGCTGCAG AAGGAGAGGG ATGAACCCGT GGCGGAGAAT GCCCAGCTGC AGAAGGAGAG 60

GGATGACGCC GTGGCGGAGA ATGCCCAGCT GCAGAAGGAG AGGGATGACG CCGTGGCGGA 120

GAATGCCCAG CTGCAGAAGG AGAGGGATGA CGCCGTGGCG GAGAATGCCC AGCTGCAGAA 180

GGAGAGGGAT GACGCCGTGG CGGAGAATGC CCAGCTGCAG AAGGAGAGGG ACGAAGCCGT 240

GGCGGAGAAT GCCCAGCTGC AGAGGGAGAG GGATGACGCC GTGGCGGAGG ATGCCCAGCT 300

GCAGAAGGAG AGGGATGAAG CCGTGGCGGA GAATGCCCAG CTGCAGAGGG AGAGGGATGA 360

AGCCGTGGCG GAGAATGCCC AGCTGCAGAA GGAGAGGGAT GACGTCGTGG CGGAGAATGC 420

CCAGCTGCAG AAGGAGAGGG ATGACGCCG: GGCGGA 456

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2446 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGAAGGCCGT TGATCCTTTT CAGGGAACGA CACCGCCGCC CTATAAATGG CAAGAAATGA 60

CTGGATCTGA GGCGGCAGCC GGCTCGCTTT GTGTACCCAG CCTTGCTGAG GTGGCCGGCG 120

GTGTGTTTGC CGTTGCTGAA GCTCAGCGCA GTGAAAGGGA CGAAGCCTGC GGCCATGCTG 180

CGATTGCAAC AACGCACATT GAGACGGGCG GTCGTGGCTC AAAGGCGATC TCGGCGATGG 240

ATGCAGGCGT TTTTCTCGTA GAACTTGTGG ATGCCGCCAG TGGTACGATC AGGACACGAG 300

AAAAGATGCA GCCAACGACA ATTGTGAGCG GCGACACTAT CTACATGGCC CTTGGGGACT 360

ACGAGAAGAA GACGTCTGGG GGTCGGGCTG CCGATGCAGA TGGCTGGAGG CTTTTACTGA 420

TGAGGGGAAC TCTCACTGAG GATGGTGGGC AGAAGAAAAT CATGTGGGGT GATATCCGTG 480

CAGTGGACCC TGTGGCCATC GGGCTTACTC AATTCCTGAA GAGGGTGATC GGTGGCGGAG 540

GATCGGGTGT TGTGACGAAG AACGGTTACC TTGTGCTTCC CATGCAGGCA GTAGAAAAGG 600

ATGGAAGGAG TGTTGTACTG TCCATGCGTT TCAACATGCG TATAGAAGCA TGCGAGCTCT 660

CGTCCGGTAC GACAGGTAGT AACTGCAAGG AACCATCCAT CGCGAATTTG GAAGGAAATC 720

TAATTTTAAT TACTTCTTGC GCTGCCGGCT ACTACGAAGT ATTCAGGTCC CTTGACTCTG 780

GGACAAGTTG GGAAATGAGT GGTAGGCCAA TTAGTCGCGT GTGGGGCAAC TCGTATGGTC 840

GAAAAGGGTA TGGCGTTCGC TGTGGCCTCA CCACCGTAAC CATTGAGGGA AGGGAAGTGC 900

TGCTTGTTAC CACGCCAGTG TATTTGGAGG AGAAAAATGG TAGGGGTCGG CTTCATCTTT 960

GGGTGACGGA CGGTGCACGT GTGCATGATG CTGGGCCGAT ATCCGATGCA GCTGATGACG 1020

CTGCTGCCAG TTCCCTGTTG TATAGCAGTG GGGGCAATCT GATTTCGCTG TACGAGAATA 1080

AGAGTGAGGG GTCATACGGT CTTGTTGCTG TGCACGTGAC TACGCAGCTG GAGCGGATAA 1140

AGACTGTGTT GAAGAGGTGG CAGGAGTTGG ATGAAGCCCT AAGAACGTGC AGATCCACTG 1200

CCACTATCGA CCCGGTGAGA AGGGGCATGT GTATTCGTCC CATTCTTACT GACGGGCTTG 1260

TTGGCTATTT GTCTGGTCTG TCGACTGGGA GTGAGTGGAT GGACGAGTAC CTCTGCGTGA 1320

ACGCAACTGT TCATGGGACG GTGAGAGGGT TCTCCAATGG AGTGACGTTT GAAGGACCCG 1380

GAGCAGGGGC GGGGTGGCCT GTTGCCCGAA GTGGACAGAA TCAACCGTAC CATTTCTTAC 1440

ACAAAACGTT CACTCTAGTG GTGATGGCGG TCATCCACGA TAGGCCGAAG AAACGCACCC 1500

CCATTCCTTT GATTCGTGTG GTGATGGATG ACAATGACAA GACTGTGCTA TTTGGTGTGT 1560

TTTACACCCA TGATGGGAGG TGGATGACTG TAATTCATAG TGGCGGTAGA CAAATACTTT 1620

CAACAGGGTG GGACCCAGAA AAACCGTGTC AGGTAGTGCT GCGACACGAC ACGGGCCATT 1680

GGGATTTCTA CGTTAACGCG AGGAAGGCTT ACTTTGGCAC CTACAAGGGT CTCTTCTCCA 1740

AACAAACAGT ATTTCACACA TCCAATTCCA CGGGGAGAGT GGGGAAGTTG CAGAGTCCAG 1800

CCATTTGTCA CTCTTCAACG CCCGTTTGTA TAACCGAAGA CTCAATTCCA AGCATCTAAG 1860

ATGGCTCATG GTCGGCGAGA CAGGCCCAAA ATACGATGAT GGCAGCTCTT ATTCTGCGAC 1920

TGCGTCCGAG GAAGGAAGCA GAGGTGGCAG CTCCATGCCC GCGGGTACGT CCGAGGAAGG 1980

AAGCAGAGGT GGCAGCTCCA TGCCTGCGGG TACGTCCGAG GAAGGAAGCA GAGGACGCAG 2040

CTCCATGCCT GCGGGTACGT CCGAGGAAGG AAGCAGAGGA GGCAGCTCCA TGCCTGCGGG 2100

TACGTCCGAG GAAGGAAGCA GAGGTGGCAG CTCCATGCCT GCGGGCACTT CCGAACAAGG 2160

AAGCAGAAGT GGCANCTCCA TGCCTTCGGG CTCTTCCGAA GAAGGAAGCA GAAGAGGCCG 2220

CTCCCTGCCT TCGGGTTCTT CCGAAGGAAG GAAGCAGAGG AGGCCCTCCC TGCCTGCGGG 2280

TTCTTCCGAA GAAGGAAACA GAAGTGGCNC TCCATGCCCG CGGGTTCTTC CGAGGAAGGA 2340

ACCAGAAGAA GCNCTCCCTG CCCGCNGGTT CNTCCNAAGA AAGAAACANA AGTTGGCCNC 2400

TCCCNGCCCC NNGTTTCTTC CNAANGAAAG AAACAAAAGT GGCCCC 2446

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 345 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGGTACGTCC GAGGAAGGAA GCAGAGGTGG CAGCTCCATG CCTGCGGGTA CGTCCGAGGA 60

AGGAAGCAGA GGTGTCAGCT CCATGCCTGC GGGTACGTCC GAGGAAGGAA ACAGAGGAGG 120

CAACTCCATG CCTGCGGGTA CGTCCGAGGA AGGAAGCAGA GGTGGCAGCT CCATGCCTTC 180

GGGCACGTCC GAGGAAGGAA GCAGAGGTGG CAGCTCCATG CCTTCGGGTA CGTCCGAGGA 240

AGGAAGCAGA GGAGGCAGCT CCATGCCTGC GGGTACGTCC GAGGAAGGAA GCAGAGGTGG 300

CAGCTCCATG CCCGCGGGTA CGTCCGAGGA AGGAAGCAGA GGCCG 345

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 835 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGCACGAGCT GTACTATATT GTAGGAGAGC AGCCATGGGT ATCGTTCGCA GCCGCCTGCA 60

TAAACGCAAG ATCACCGGTG GAAAGACGAA GATCCACCGG AAGCGCATGA AGGCCGAACT 120

CGGCCGTCTT CCCGCGCACA CGAAGCTTGG CGCCCGCCGC GTGAGTCCCG TCCGCGCCCG 180

CGGTGGGAAC TTCAAGCTCC GCGGTCTTCG CCTGGACACC GGCAATTTTG CGTGGAGCAC 240

AGAAGCCATT GCTCAGCGGG CCCGTATCCT CGACGTTGTG TACAACGCCA CTTCTAACGA 300

GCTGGTGCGC ACGAAGACGC TTGTGAAGAA CTGCATTGTT GTGGTGGACG CCGCGCCCTT 360

CAAGTTATGG TACGCGAAGC ACTACGGTAT CGACCTTGAG CCGCGAAGAG CAAGAAGACG 420

CTGCAGAGCA CGACGGAGAA GAAGAAGTCG AAGAAGACCT CACACGCCAT GACTGAGAAG 480

TACGACGTCA AGAAGGCCTC CGACGAGCTG AAGCGCAAGT GGATGCTCCG CCGCGAGAAC 540

CACAAGATTG AGAACGCAGT TGCTGATCAG CTCAAGGAGG GCCGTCTGCT CGGCCGCATC 600

ACGAGCCGCC CTGGCCAGAC AGCCCGCGCC GATGGTGCAC TGCTGGAGGG CGCCGAACTG 660

CAGTTCTATC TGAAGAAGCT CGAGAAGAAG AAGCGGTAGA GAAGGATGTT CGGGAGACGG 720

GAGGAGGCGC CACCACCACC ACTCATGGTG ATGCACCCAC TACCTACTTT GTTTTCATTT 780

TTTGTTTTAC CTCTAATTTT TTAGGCCAGA GGGGGGGAAA AAAAAAAAAA AAAAA 835

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 555 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCACGAGAA AAAAGAAAAC AAACAAATAA AATCAAAAAC AGTAAATCCA TCACTTCAAC 60

AATGAGCATT GAGAGCGCCT TTTACGCCTT TGCCTCCTTT GGTGGTGCGC CCACGAAAGA 120

GATGGACAAT GCTCACTTCT CCAAGATGCT GAAGGAGACG AAGGTCATTG GAAAGCAATT 180

CACCAGCACC GACGCCGATC TTCTCTTCAA CAAAGTGAAG GCAAAGGGAG CCCGCAAAAT 240

TACATTGTCG GATTTTGTTG ACAAGGCTGT TCCTGAGATT GCATCAAAGT TAAAGAAGTC 300

CGCGGAGGAA TTGATCGCAG ATATTTCAAG TTGCTCTCCC GAGGCACGCC CAACCAAGGC 360

CGATGCAGTT AAGTTCCACG ACGATAAGAA CATGTACACT GGTGTCTACA AGGCCGGCGG 420

GCCAACAAAC GTGGATCGCA ACTCCGGCTC CTTTCAGGT GTCGTGGATC GCCGTGTGGC 480

GCAGACTGAC GTTCGTGGCA CGACTGCTTC CCAGAAGTAA AGAGGGAAAC GAAATGGAAA 540

AAAAAAAAAA AAAAA 555

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 936 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGCACGAGAG CTCTCTTCGT CAGTCATGAC GCTCGGGAAG AACAAGCGCA TCAGCAAGGG 60

CGGCAAGCGC GGCAAGAAGA AGACCCAGGA GACGATGAGC CGCAAGGAGT GCTACGATGT 120

GGTTGCCCCC AAGAACTTTG AGGTGCGCCA GTTTGGCAAG ACCATCTGCA ACAAGACCCA 180

GGGCACAAAG ATCGCGGCGG ACTACCTGCG CGGGGCGCGTG TACGAAAGCA ACCTTGCGGA 240

TCTGAACAAG ACGCAAGGCG ACGACGACGC CTACCGCAAG GTGAAGTTTG TTGTGCAGGA 300

GGTGCAGGGC CGCAACCTGC TTACGCAGTT CCACAGCATG GAAATGACAT CTGACCGCGT 360

GTACTTTTTG CTGCGCAAGT GGTGCACGAC GATCGAGGCG GCAGTGGAGA CGAAGACTGC 420

GGACGGCTAC ACCCTGCGCC TCTTCGTGAT TGCCTTCACG AAGAAGCAGA GCAACCAGCT 480

GTCGAAGAAC TGCTATGCCA AGACGCGCCT GGTGAAGTGG GTGCGCCATC GCATCACGAA 540

CCTCATCCGC CAGCGCCTGT CGAAGGTGAA CATCAACGAG GCGGTGACGC TGCTGACACG 600

CAACATCCTG CGCGATCGTC TGGCAAAGCG CTGCAACCCC ATCGTGCCGC TGCGCGATCT 660

CCGCATCCGC AAGGTGAAGG TGGTCCGCAC CCCCCGGTTT TGACGCCCAG GCGCTTCTGA 720

ATGCACACGG CGAGATCCCC GCCTCGGCTG AGGGTGAGGC ACGCGTCGTC GAGGAAGCCC 780

AAGAGGCTCC CGCCGCTGAA GCCACAGCCI AAGCCTTCCA TGTGGAGGAA GGATGTGTGA 840

TGTGAAAGCT CTTTGTTCTT TTTTCTTTCT ATTTTGAAAC GGTGATTCCG CATATATATA 900

TTAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA 936

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 581 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTACTATATI GTTGCTATTA ACACACTGTT AGGAACGCGA AACCATGCAG ATCTTCGTGA 60

AGACACTGAC GGGCAAGACG ATCGCGCTCG AGGTGGAATC CAGCGACACC ATTGAGAACG 120

TGAAGGCGAA GATCCAGGAC AAGGAGGGCA TTCCGCCGGA CCAGCAGCGC CTGATCTTCG 180

CTGGCAAGCA GCTGGAGGAC GGCCGCACGC TCGCAGACTA CAACATCCAG AAGGAGTCCA 240

CGCTGCACCT TGTGCTGCGC CTGCGCGGTG GTGTGATGGA GCCGACACTT GAGGCCCTGG 300

CGAAGAAGTA CAACTGGGAG AAGAAGGTAT GCCGCCGCTG CTACGCCCGT CTGCCGGTGC 360

GTGCGTCCAA CTGCCGCAAG AAGGCATGTG GCCACTGCTC CAACCTCCGC ATGAAGAAGA 420

AGCTGCGGTA GTCTGCGATG CTGTGGACCG ACGCATTGAA ATACACACCG TCTTCGGCGT 480

TCCTTTTTTT TATATGTCTT TTTTTTATT GAGAAGATGT CTTGTTTGTT GTTGTTTTTT 540

TTTCAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 581

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Leu Pro Pro Leu Leu Pro Ser Ser Asp Val Pro Glu Gly Met Glu Leu
1 5 10 15

Pro Pro Leu Leu Pro Ser Ser Asp Ile Pro Glu Gly Met Glu
20 25 30

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Cys Leu Leu Cys Phe Leu Pro Arg Thr Cys Pro Lys Ala Trp Ser
1 5 10 15

Cys Leu Leu Cys Phe Leu Pro Arg Thr Tyr Pro Lys Ala Trp Ser Cys
20 25 30

His Leu Cys Phe Leu Pro Arg Thr Tyr Pro Arg Ala Trp Ser Cys His
35 40 45

Leu Cys Phe Leu Pro Arg Thr Cys Pro Lys Ala Trp Ser Cys His Leu
50 55 60

Cys Phe Leu Pro Arg Thr Tyr Pro Arg Ala Trp Ser Cys His Leu Cys
65 70 75 80

Phe Leu Pro Arg Thr Tyr Pro Arg Val Trp
85 90

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Ala Ser Ser Ala Ser Phe Leu Gly Arg Ala Arg Arg His Gly Ala
1 5 10 15

Ala Ser Ser Ala Ser Phe Leu Gly His Thr Arg Arg His Gly Ala Ala
20 25 30

Thr Ser Ala Ser Phe Leu Gly Arg Thr Arg Gly His Gly Ala Ala Thr
35 40 45

Ser Ala Ser Phe Leu Gly Arg Ala Arg Arg His Gly Ala Ala Thr Ser
50 55 60

Ala Ser Phe Leu Gly Arg Thr Arg Gly His Gly Ala Ala Thr Ser Ala
65 70 75 80

Ser Phe Leu Gly Arg Thr Arg Gly His Gly
85 90

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser Val Pro Gly Lys Arg Leu Arg Asn Ser His Gly Lys Ser Leu Arg
1 5 10 15

Asn Val His Gly Lys Arg Pro Lys Asn Glu His Gly Lys Arg Leu Arg
20 25 30

Ser Val Pro Asn Glu Arg Leu Arg
35 40

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Glu Ala Glu Glu Leu Ala Arg Gln Glu Ser Glu Glu Arg Ala Arg Gln
1 5 10 15

Glu Ala Glu Glu Arg Ala Trp Gln Glu Ala Glu Glu Arg Ala Gln Arg
20 25 30

Glu Ala Glu Glu Arg Ala Gln Arg
35 40

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ser Trp Gln Ser Gln Ser His Gln Gln Gln Val Pro Thr Cys Ala Arg
1 5 10 15

Gln Ser Arg Ser His Gln Gln Gln Ala Pro Lys Trp Ser Trp Gln Ser
20 25 30

Gln Ser His Gln Gln Gln Val Pro Thr Cys Ala Arg Gln Ser Arg Ser
35 40 45

His Gln Gln Gln Val Pro Thr Trp
50 55

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Arg Gly Arg Ala Lys Ala Thr Asn Ser Arg Cys Arg Arg Val Arg
1 5 10 15

Gly Arg Ala Glu Ala Thr Ser Ser Arg Arg Arg Ser Gly Arg Gly Arg
20 25 30

Ala Lys Ala Thr Ser Ser Arg Cys Arg Pro Val Arg Gly Arg Ala Glu
35 40 45

Ala Thr Asn Ser Arg Cys Arg Arg
50 55

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Val Val Ala Glu Pro Lys Pro Pro Thr Ala Gly Ala Asp Val Cys Ala
1 5 10 15

Ala Glu Pro Lys Pro Pro Ala Ala Gly Ala Glu Val Val Val Ala Glu
20 25 30

Pro Lys Pro Pro Ala Ala Gly Ala Asp Val Cys Ala Ala Glu Pro Lys
35 40 45

Pro Pro Thr Ala Gly Ala Asp Val
50 55

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Pro Pro Ala Lys Ala Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Val Leu Gln Lys Glu Arg Asp Glu Ala Val Ala Glu Asn Ala Gln Leu
1 5 10 15

Gln Lys Glu Arg Asp Asp Ala Val Ala Glu Asn Ala Gln Leu Gln Lys
20 25 30

Glu Arg Asp Asp Ala Val Ala Glu Asn Ala Gln Leu Gln Lys Glu Arg
35 40 45

Asp Asp Ala Val Ala Glu Asn Ala Gln Leu Gln Lys Glu Arg Asp Asp
50 55 60

Ala Val Ala Glu Asn Ala Gln Leu Gln Lys Glu Arg Asp Glu Ala Val
65 70 75 80

Ala Glu Asn Ala Gln Leu Gln Arg Glu Arg Asp Asp Ala Val Ala Glu
85 90 95

Asp Ala Gln Leu Gln Lys Glu Arg Asp Glu Ala Val Ala Glu Asn Ala
100 105 110

Gln Leu Gln Arg Glu Arg Asp Glu Ala Val Ala Glu Asn Ala Gln Leu
115 120 125

Gln Lys Glu Arg Asp Asp Val Val Ala Glu Asn Ala Gln Leu Gln Lys
130 135 140

Glu Arg Asp Asp Ala Val Ala
145 150

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Arg Arg Arg Gly Met Lys Pro Trp Arg Arg Met Pro Ser Cys Arg
1 5 10 15

Arg Arg Gly Met Thr Pro Trp Arg Arg Met Pro Ser Cys Arg Arg Arg
20 25 30

Gly Met Thr Pro Trp Arg Arg Met Pro Ser Cys Arg Arg Arg Gly Met
35 40 45

Thr Pro Trp Arg Arg Met Pro Ser Cys Arg Arg Arg Gly Met Thr Pro
50 55 60

Trp Arg Arg Met Pro Ser Cys Arg Arg Arg Gly Thr Lys Pro Trp Arg
65 70 75 80

Arg Met Pro Ser Cys Arg Gly Arg Gly Met Thr Pro Trp Arg Arg Met
85 90 95

Pro Ser Cys Arg Arg Arg Gly Met Lys Pro Trp Arg Arg Met Pro Ser
100 105 110

Cys Arg Gly Arg Gly Met Lys Pro Trp Arg Arg Met Pro Ser Cys Arg
115 120 125

Arg Arg Gly Met Thr Ser Trp Arg Arg Met Pro Ser
130 135 140

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Tyr Val Arg Gly Arg Lys Gln Arg Trp Gln Leu His Ala Phe Gly
1 5 10 15

Tyr Val Arg Gly Arg Lys Gln Arg Trp Gln Leu His Ala Phe Gly Tyr
20 25 30

Val Arg Gly Arg Lys Gln Arg Arg Gln Leu His Ala Cys Gly Tyr Val
35 40 45

Arg Gly Arg Lys Gln Arg Trp Gln Leu His Ala Cys
50 55 60

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Thr Ser Glu Glu Gly Ser Arg Gly Gly Ser Ser Met Pro Ser Gly
1 5 10 15

Thr Ser Glu Glu Gly Ser Arg Gly Gly Ser Ser Met Pro Ser Gly Thr
20 25 30

Ser Glu Glu Gly Ser Arg Gly Gly Ser Ser Met Pro Ala Gly Thr Ser
35 40 45

Glu Glu Gly Ser Arg Gly Gly Ser Ser Met Pro Ala
50 55 60

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Val Arg Pro Arg Lys Glu Ala Glu Val Ala Ala Pro Cys Leu Arg Val
1 5 10 15

Arg Pro Arg Lys Glu Ala Glu Val Ala Ala Pro Cys Leu Arg Val Arg
20 25 30

Pro Arg Lys Glu Ala Glu Glu Ala Ala Pro Cys Leu Arg Val Arg Pro
35 40 45

Arg Lys Glu Ala Glu Val Ala Ala Pro Cys Leu Arg
50 55 60

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 639 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Asp Ala Ser Val Val Asp Leu Gly Gly Glu Ala His Gly Thr His Tyr
1 5 10 15

Ala Phe Leu Pro Asp Val Ile Lys Gly Ile Ala Gln Glu Glu Leu Tyr
20 25 30

Leu Glu Asp Asp Ala Tyr Phe Gln Glu Leu Leu Ala Arg Tyr Lys Glu
35 40 45

Leu Val Pro Val Gly Ala Glu Pro Thr Glu Pro Arg Ala Lys Gln Leu
50 55 60

Arg Glu Gln Met Arg Ile Arg Ala Gly Gln Leu Ala Val Asp Thr Arg
65 70 75 80

Lys Leu His Ala Ala Glu Glu Arg Ala Ala Ser Arg Met Ala Thr Leu
85 90 95

Tyr Pro Phe Val Gly Ser Ala Pro Leu Gly Val Ala Leu Trp Asn Ile
100 105 110

Pro Val Glu Ala Asp Glu Glu Phe Cys Ala Leu Leu Leu Lys Arg Glu
115 120 125

Glu Ala Leu Ala Gly Lys Ser Gly Ser Val His Glu Val Glu Ser Ala
130 135 140

Leu Ser Ala Arg Ala Glu Ala Met Ala Lys Ala Val Leu Glu Glu Glu
145 150 155 160

Glu Ala Leu Ala Ala Ala Phe Pro Phe Leu Gly Arg Ser Val Lys Gly
165 170 175

Ala Pro Leu Arg Glu Leu Ala Leu Met Ser Asp Pro Asn Phe Ala Glu
180 185 190

Leu Ala Thr Arg His Ala Gln Glu Ala Thr Ser Gly Asp Ala Ala Gly
195 200 205

Ile Leu Arg Leu Glu Gln Glu Leu Arg Asp Gln Ala Cys Arg Ile Ala
210 215 220

Arg Glu Val Arg Val Ala Arg Arg Leu Asp Ala Xaa Arg Asn Glu Asp
225 230 235 240

Leu His Glu Arg Tyr Pro Phe Leu Pro Glu Glu Pro Val Arg Gly Ile
245 250 255

Leu Leu Gly Ala Val Arg Pro Val Gln Gln Pro Ala Phe Arg Glu Leu
260 265 270

Ser Asn Lys Leu Asp Glu Gln Arg Arg Asp Pro Thr Arg Asn Ala Ala
275 280 285

Ala Ile Arg Thr Thr Glu Glu Gln Met Thr Ala Leu Val Val Arg Leu
290 295 300

Ala Glu Glu Arg Ala Glu Ala Thr Glu Arg Ala His Glu Gln Tyr Pro
305 310 315 320

Phe Leu Pro Arg Arg Val Leu Gly Val Arg Leu Gly Asp Ile Ser Leu
325 330 335

Gln Glu Asp Asp Val Leu Ser Gln Leu Ala Arg Arg Arg Val Arg Gln
340 345 350

Leu Arg Asn Ser Lys Thr Ala Ile Asp Ala His Ala Thr Glu Glu Glu
355 360 365

Met Ile Arg Arg Ala Glu Glu Leu Ala Arg Asn Val Lys Leu Val Asp
370 375 380

Ala Tyr Arg Gly Asn Gly Asn Glu Tyr Val Arg Ala Cys Asn Pro Phe
385 390 395 400

Leu Val Tyr Glu Asp Arg Lys Cys Val Leu Leu Ser Glu Leu Pro Leu
405 410 415

Ala Gly Gly Asp Val Tyr Gln Gly Leu Phe Arg Asp Tyr Leu Thr Ala
420 425 430

Leu Glu Asp Ala Glu Ala Asn Ala Pro Arg Ile Ala Glu Leu Glu Asn
435 440 445

Ala Leu Arg Ser Arg Ala Asp Glu Leu Ala Leu Glu Val Cys Glu Arg
450 455 460

Asp Ala Arg Leu Leu His Tyr Ser Phe Leu Ser Ala Gln Asp Val Pro
465 470 475 480

Gly Trp Ser Glu Ala Leu Leu His Asp Ala Glu Phe Gln Gln Leu Arg
485 490 495

Glu Arg Tyr Glu Glu Leu Ser Lys Asp Pro Gln Gly Asn Ala Glu Ala
500 505 510

Leu Arg Glu Leu Glu Asp Ala Met Glu Ala Arg Ser Arg Ala Ile Ala
515 520 525

Glu Ala Leu Arg Thr Ala Glu Xaa Thr Asn Xaa Thr Glu Gln Ala Arg
530 535 540

Leu Lys Thr Pro Ser Gln Ala Gly Ser Gly Val Ser Ala Gly Asp Arg
545 550 555 560

Met His Gly Ser Glu His Ala Asp Leu Ala His Glu Gly Gly Ser Thr
565 570 575

Ala Gly Gly Thr Met Arg Gly Ala Glu Ser Val Ser Lys Ser Ser Gly
580 585 590

Lys His Ser Xaa Arg Ser Val Ser His Ala Ser Val Val Asp Leu Gly
595 600 605

Gly Glu Ala His Gly Thr His Tyr Ala Phe Leu Pro Asp Val Ile Lys
610 615 620

Gly Ile Ala Gln Glu Glu Leu Tyr Leu Glu Asp Asp Ala Tyr Phe
625 630 635

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 231 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Arg Ala Val Leu Tyr Cys Arg Arg Ala Ala Met Gly Ile Val Arg
1 5 10 15

Ser Arg Leu His Lys Arg Lys Ile Thr Gly Gly Lys Thr Lys Ile His
20 25 30

Arg Lys Arg Met Lys Ala Glu Leu Gly Arg Leu Pro Ala His Thr Lys
35 40 45

Leu Gly Ala Arg Arg Val Ser Pro Val Arg Ala Arg Gly Gly Asn Phe
50 55 60

Lys Leu Arg Gly Leu Arg Leu Asp Thr Gly Asn Phe Ala Trp Ser Thr
65 70 75 80

Glu Ala Ile Ala Gln Arg Ala Arg Ile Leu Asp Val Val Tyr Asn Ala
85 90 95

Thr Ser Asn Glu Leu Val Arg Thr Lys Thr Leu Val Lys Asn Cys Ile
100 105 110

Val Val Val Asp Ala Ala Pro Phe Lys Leu Trp Tyr Ala Lys His Tyr
115 120 125

Gly Ile Asp Leu Asp Ala Ala Lys Ser Lys Lys Thr Leu Gln Ser Thr
130 135 140

Thr Glu Lys Lys Lys Ser Lys Lys Thr Ser His Ala Met Thr Glu Lys
145 150 155 160

Tyr Asp Val Lys Lys Ala Ser Asp Glu Leu Lys Arg Lys Trp Met Leu
165 170 175

Arg Arg Glu Asn His Lys Ile Glu Lys Ala Val Ala Asp Gln Leu Lys
180 185 190

Glu Gly Arg Leu Leu Ala Arg Ile Thr Ser Arg Pro Gly Thr Ala Arg
195 200 205

Ala Asp Gly Ala Leu Leu Glu Gly Ala Glu Leu Gln Phe Tyr Leu Lys
210 215 220

Lys Leu Glu Lys Lys Lys Arg
225 230

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Arg Glu Lys Arg Lys Gln Thr Asn Lys Ile Lys Asn Ser Lys Ser
1 5 10 15

Ile Thr Ser Thr Met Ser Glu Glu Ser Ala Phe Tyr Ala Phe Ala Ser
20 25 30

Phe Gly Gly Ala Pro Thr Lys Glu Met Asp Asn Ala His Phe Ser Lys
35 40 45

Met Leu Lys Glu Thr Lys Val Ile Gly Lys Gln Phe Thr Ser Thr Asp
50 55 60

Ala Asp Leu Leu Phe Asn Lys Val Lys Ala Lys Gly Ala Arg Lys Ile
65 70 75 80

Thr Leu Ser Asp Phe Val Asp Lys Ala Val Pro Glu Ile Ala Ser Lys
85 90 95

Leu Lys Lys Ser Ala Glu Glu Leu Ile Ala Asp Ile Ser Ser Cys Ser
100 105 110

Pro Glu Ala Arg Ala Thr Lys Ala Asp Ala Val Lys Phe His Asp Asp
115 120 125

Lys Asn Met Tyr Thr Gly Val Tyr Lys Ala Gly Gly Pro Thr Asn Val
130 135 140

Asp Arg Asn Ser Gly Ser Leu Ser Gly Val Val Asp Arg Arg Val Ala
145 150 155 160

Gln Thr Asp Val Arg Gly Thr Thr Ala Ser Gln Lys
165 170

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(x') SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ala Arg Glu Leu Ser Ser Ser Val Met Thr Leu Gly Lys Asn Lys Arg
1 5 10 15

Ile Ser Lys Gly Gly Lys Arg Gly Lys Lys Lys Thr Gln Glu Thr Met
20 25 30

Ser Arg Lys Glu Trp Tyr Asp Val Val Ala Pro Lys Asn Phe Glu Val
35 40 45

Arg Gln Phe Gly Lys Thr Ile Cys Asn Lys Thr Gln Gly Thr Lys Ile
50 55 60

Ala Ala Asp Tyr Leu Arg Gly Arg Val Tyr Glu Ser Asn Leu Ala Asp
65 70 75 80

Leu Asn Lys Thr Gln Gly Asp Asp Asp Ala Tyr Arg Lys Val Lys Phe
85 90 95

Val Val Gln Glu Val Gln Gly Arg Asn Leu Leu Thr Gln Phe His Ser
100 105 110

Met Glu Met Thr Ser Asp Arg Val Tyr Phe Leu Leu Arg Lys Trp Cys
115 120 125

Thr Thr Ile Glu Ala Ala Val Glu Thr Lys Thr Ala Asp Gly Tyr Thr
130 135 140

Leu Arg Leu Phe Val Ile Ala Phe Thr Lys Lys Gln Ser Asn Gln Leu
145 150 155 160

Ser Lys Asn Cys Tyr Ala Lys Thr Arg Leu Val Lys Trp Val Arg His
165 170 175

Arg Ile Thr Asn Leu Ile Arg Gln Arg Leu Ser Lys Val Asn Ile Asn
180 185 190

Glu Ala Val Thr Leu Leu Thr Arg Asn Ile Leu Arg Asp Arg Leu Ala
195 200 205

Lys Arg Cys Asn Pro Ile Val Pro Leu Arg Asp Leu Arg Ile Arg Lys
210 215 220

Val Lys Val Val Arg Thr Pro Arg Phe
225 230

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu
1 5 10 15

Val Glu Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp
20 25 30

Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
35 40 45

Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu
50 55 60

Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Val Met Glu Pro
65 70 75 80

Thr Leu Glu Ala Leu Ala Lys Lys Tyr Asn Trp Glu Lys Lys Val Cys
85 90 95

Arg Arg Cys Tyr Ala Arg Leu Pro Val Arg Ala Ser Asn Cys Arg Lys
100 105 110

Lys Ala Cys Gly His Cys Ser Asn Leu Arg Met Lys Lys Lys Leu Arg
115 120 125

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Arg Leu Pro Pro Leu Leu Pro Ser Ser Asp Val Pro Glu Gly Met Glu
1 5 10 15

Leu Pro Pro Leu Leu Pro Ser Ser Asp Ile Pro Glu Gly Met Glu Leu
20 25 30

Pro Pro Leu Leu Pro Ser Ser Asp Val Pro Ala Gly Met Glu Leu Thr
35 40 45

Pro Leu Leu Pro Ser Ser Asp Val Pro Glu Gly Met Glu Leu Pro Pro
50 55 60

Leu Leu Pro Ser Ser Asp Val Pro Ala Gly Met Glu Leu Pro Pro Leu
65 70 75 80

Xaa Pro Ser Ser Asp Val Pro Ala Gly Met Glu Leu Pro Pro Leu Leu
85 90 95

Pro Ser Ser Asp Val Pro Ala Xaa Ile Glu Leu Pro Pro Leu Ile Ser
100 105 110

Xaa Leu Gly Arg Thr Xaa Arg Xaa Gly Asp Xaa Ser Ser Xaa Ser Cys
115 120 125

Leu Gly Arg Xaa Xaa Arg Xaa Arg Xaa Ala Pro Leu Xaa Pro Xaa Ser
130 135 140

Glu
145

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 186 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Glu Lys Glu Arg Arg Phe Pro Thr Lys Thr Ala Arg Ala Asp Pro Thr
1 5 10 15

Thr Thr Lys Gln Leu Ile Ile Arg Ala Leu Gln Asn Ile Ser Leu Ala
20 25 30

Phe Gly Ile Glu Pro Ser Ser Thr Val Lys Tyr Ala Glu Ser Thr Gln
35 40 45

Glu Glu Asn Gly Lys Arg Ser Gln Ser Glu Ala Glu Glu Arg Ala Arg
50 55 60

Arg Glu Ala Glu Glu Arg Ala Arg Arg Glu Ala Glu Glu Arg Ala Gln
65 70 75 80

Arg Glu Ala Glu Glu Arg Ala Gln Arg Glu Ala Glu Glu Arg Ala Arg
85 90 95

Arg Glu Ala Glu Lys Arg Ala Arg Arg Glu Ala Lys Glu Arg Ala Trp
100 105 110

Gln Glu Ala Glu Glu Arg Ala Gln Arg Glu Ala Glu Glu Arg Ala Arg
115 120 125

Arg Glu Ala Glu Glu Arg Ala Arg Arg Glu Val Glu Glu Arg Ala Arg
130 135 140

Gln Glu Ala Glu Glu Leu Ala Arg Gln Glu Ser Glu Glu Arg Ala Arg
145 150 155 160

Gln Glu Ala Glu Glu Arg Ala Trp Gln Glu Ala Glu Glu Arg Ala Gln
165 170 175

Arg Glu Ala Glu Glu Arg Ala Gln Arg Ala
180 185

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Arg Gly Arg Ala Lys Ala Thr Asn Ser Arg Cys Arg Arg Val Arg
1 5 10 15

Gly Arg Ala Glu Ala Thr Ser Ser Arg Arg Arg Ser Gly Arg Gly Arg
20 25 30

Ala Lys Ala Thr Ser Ser Arg Cys Arg Arg Val Arg Gly Arg Val Glu
35 40 45

Ala Thr Asn Ser Arg Cys Arg Arg Gly Arg Gly Arg Ala Lys Val Thr
50 55 60

Ser Ser Arg Xaa Arg Arg Val Xaa Gly Arg Xaa Xaa Xaa Thr Ser Xaa
65 70 75 80

Arg Xaa Arg Arg Xaa Arg Gly Arg Xaa Xaa Val Thr Ser Arg Arg Xaa
85 90 95

Arg Arg Xaa Xaa Gly Arg Gly Asp Val Thr
100 105

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45

Ser Ile Pro Val Glu Ile Asp Ile Arg Asn Gln Asp Phe Ser Phe Leu
1 5 10 15

Asp Pro Ala Pro Glu Gly Ile Pro Ile Gln Asp Ile His Leu Met Gly
20 25 30

Asp Ser Ala Phe Ala Ala Ser Ala Arg Glu Arg Met Lys Leu Lys Arg
35 40 45

Asn Pro Val Ala Asn Ala Ser Lys Ile Ser Ala Leu Glu Glu Glu Met
50 55 60

Asp Gln Arg Ala His Val Leu Ala Lys Gln Val Arg Asp Lys Glu Arg
65 70 75 80

Thr Phe Leu Asp Pro Glu Pro Glu Gly Val Pro Leu Glu Leu Leu Ser
85 90 95

Leu Asn Glu Asn Glu Ala Ser Gln Glu Leu Glu Arg Glu Leu Arg Ala
100 105 110

Leu Asn Arg Lys Pro Arg Lys Asp Ala Lys Ala Ile Val Ala Leu Glu
115 120 125

Asp Asp Val Arg Asp Glu His Thr Cys Leu Pro Arg Ser
130 135 140

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Arg Lys Met Ser Gly Thr Ser Leu Leu Ala Pro Gln Pro Glu Gly Val
1 5 10 15

Pro Val Ser Glu Leu Ser Leu Asp Leu Asp Glu
20 25

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS.
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Leu Leu Ala Leu Leu Gln Gly Leu Val Gln Leu Arg Thr Gln Ile His
1 5 10 15

Gly Val Arg Pro Ala Leu Leu Pro Glu Ser Gly Gln Phe Leu Gly Gly
20 25 30

Ser Leu Gln Leu Ala Met His Leu Leu Ala Leu Leu Gln Gly Leu Val
35 40 45

Gln Leu Arg Thr Gln Ile His Gly Val Arg Pro Ala Leu Leu Pro Glu
50 55 60

Ser Gly Gln Phe Leu Gly Gly Ser Leu Gln Leu Ala Met His Leu Leu
65 70 75 80

Ala Leu Leu Gln Gly Leu Val Gln Leu Arg Thr Gln Ile His Gly Val
85 90 95

Arg Pro Ala Leu Leu Pro Glu Ser Gly Gln Phe Leu Gly Gly Ser Leu
100 105 110

Gln Leu Ala Thr His
115

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Ser Arg Cys Cys Lys Ala Ser Ser Ser Cys Ala Arg Arg Phe Thr
1 5 10 15

Val Ser Ala Pro Leu Cys Ser Arg Arg Ala Ala Ser Ser Ser Val Val
20 25 30

Arg Phe Ser Ser Arg Cys Thr Ser Ser Arg Cys Cys Lys Ala Ser Ser
35 40 45

Ser Cys Ala Arg Arg Phe Thr Val Ser Ala Pro Leu Cys Ser Arg Arg
50 55 60

Ala Gly Ser Ser Ser Val Val Arg Phe Ser Ser Arg Cys Thr Ser Ser
65 70 75 80

Arg Cys Cys Lys Ala Ser Ser Ser Cys Ala Arg Arg Phe Thr Val Ser
85 90 95

Ala Pro Leu Cys Ser Arg Arg Ala Gly Ser Ser Ser Val Val Arg Phe
100 105 110

Ser Ser Arg Arg Thr
115

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Pro Pro Arg Ala Ala Ala Arg Pro Arg Pro Ala Ala His Ala Asp Ser
1 5 10 15

Arg Cys Pro Pro Arg Ser Ala Pro Gly Glu Arg Pro Val Pro Arg Trp
20 25 30

Phe Ala Ser Ala Arg Asp Ala Pro Pro Arg Ala Ala Ala Arg Pro Arg
35 40 45

Pro Ala Ala His Ala Asp Ser Arg Cys Pro Pro Arg Ser Ala Pro Gly
50 55 60

Glu Arg Ala Val Pro Arg Trp Phe Ala Ser Ala Arg Asp Ala Pro Pro
65 70 75 80

Arg Ala Ala Ala Arg Pro Arg Pro Ala Ala His Ala Asp Ser Arg Cys
85 90 95

Pro Pro Arg Ser Ala Pro Gly Glu Arg Ala Val Pro Arg Trp Phe Ala
100 105 110

Ser Ala Arg Asp Ala
115

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acic
(C) STRANDEDNFSS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala
1 5 10 15

Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu
20 25 30

Pro Lys Prc Ala Glu Pro Lys Ser Ala Gly Pro Lys Pro Ala Glu Pro
35 40 45

Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys
50 55 60

Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser
65 70 75 80

Ala Glu Pro Lys Pro Ala Glu Ser Lys Ser Ala Glu Pro Lys Pro Ala
85 90 95

Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Ser Lys Ser Ala Glu
100 105 110

Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro
115 120 125

Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys
130 135 140

Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Ser Lys Ser
145 150 155 160

Ala Gly Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala
165 170 175

Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu
180 185 190

Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu
195 200 205

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 263 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Arg Arg Gly Tyr Pro Arg Ser Arg Met Pro Ser Lys Glu Leu Trp Met
1 5 10 15

Arg Arg Leu Arg Ile Leu Arg Arg Leu Leu Arg Lys Tyr Arg Glu Glu
20 25 30

Lys Lys Ile Asp Arg His Ile Tyr Arg Glu Leu Tyr Val Lys Ala Lys
35 40 45

Gly Asn Val Phe Arg Asn Lys Arg Asn Leu Met Glu His Ile His Lys
50 55 60

Val Lys Asn Glu Lys Lys Lys Glu Arg Gln Leu Ala Glu Gln Leu Ala
65 70 75 80

Ala Asn Ala Xaa Lys Asp Glu Gln His Arg His Lys Ala Arg Lys Gln
85 90 95

Glu Leu Arg Lys Arg Glu Lys Asp Arg Glu Arg Ala Arg Arg Glu Asp
100 105 110

Ala Ala Ala Ala Ala Ala Ala Lys Gln Lys Ala Ala Ala Lys Lys Ala
115 120 125

Ala Ala Pro Ser Gly Lys Lys Ser Ala Lys Ala Ala Ile Ala Pro Ala
130 135 140

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala
145 150 155 160

Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala
165 170 175

Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala Lys Ala Ala Ala Ala Pro
180 185 190

Ala Lys Thr Ala Ala Ala Pro Ala Lys Ala Ala Ala Pro Ala Lys Ala
195 200 205

Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala Lys Ala Ala Ala
210 215 220

Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala Lys Ala Ala Thr Ala Pro
225 230 235 240

Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Val Gly
245 250 255

Lys Lys Ala Gly Gly Lys Lys
260

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 442 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Asp Phe Ile Trp Tyr Lys Val Val Ala Leu Leu Val Val Ile Thr Ser
1 5 10 15

Asn Gly Asp Asp Val Ser Val Tyr Thr Ala Thr Ile Lys Glu Phe Tyr
20 25 30

Arg Tyr Leu Trp Ile Phe Val Pro Val Ser Leu Phe Ser Ile Ile Ile
35 40 45

Tyr Phe Val Ser Ile Phe Cys Phe Pro Ala Ser Tyr Gly Leu Phe Phe
50 55 60

Ser Ser Phe Leu Lys Phe Gln Leu Leu Leu Asn His Lys His Pro Val
65 70 75 80

Leu Gln Pro Pro His Gln Met Val Ser Leu Lys Leu Gln Ala Arg Leu
85 90 95

Ala Ala Asp Ile Leu Arg Cys Gly Arg His Arg Val Trp Leu Asp Pro
100 105 110

Asn Glu Ala Ser Glu Ile Ser Asn Ala Asn Ser Arg Lys Ser Val Arg
115 120 125

Lys Leu Ile Lys Asp Gly Leu Ile Ile Arg Lys Pro Val Lys Val His
130 135 140

Ser Arg Ser Arg Trp Arg His Met Lys Glu Ala Lys Ser Met Gly Arg
145 150 155 160

His Glu Gly Ala Gly Arg Arg Glu Gly Thr Arg Glu Ala Arg Met Pro
165 170 175

Ser Lys Glu Leu Trp Met Arg Arg Leu Arg Ile Leu Arg Arg Leu Leu
180 185 190

Arg Lys Tyr Arg Glu Glu Lys Lys Ile Asp Arg His Ile Tyr Arg Glu
195 200 205

Leu Tyr Val Lys Ala Lys Gly Asn Val Phe Arg Asn Lys Arg Asn Leu
210 215 220

Met Glu His Ile His Lys Val Lys Asn Glu Lys Lys Lys Glu Arg Gln
225 230 235 240

Leu Ala Glu Gln Leu Ala Ala Lys Arg Leu Lys Asp Glu Gln His Arg
245 250 255

His Lys Ala Arg Lys Gln Glu Leu Arg Lys Arg Glu Lys Asp Arg Glu
260 265 270

Arg Ala Arg Arg Glu Asp Ala Ala Ala Ala Ala Ala Ala Lys Gln Lys
275 280 285

Ala Ala Ala Lys Lys Ala Ala Ala Pro Ser Gly Lys Lys Ser Ala Lys
290 295 300

Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala
305 310 315 320

Pro Pro Ala Lys Thr Ala Ala Ala Pro Ala Lys Ala Ala Ala Pro Ala
325 330 335

Lys Ala Ala Ala Pro Pro Ala Lys Ala Ala Ala Pro Pro Ala Lys Thr
340 345 350

Ala Ala Pro Pro Ala Lys Thr Ala Ala Pro Pro Ala Lys Ala Ala Ala
355 360 365

Pro Pro Ala Lys Ala Ala Ala Pro Pro Ala Lys Ala Ala Ala Pro Pro
370 375 380

Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys
385 390 395 400

Ala Ala Ala Pro Pro Ala Lys Ala Ala Ala Pro Pro Ala Lys Ala Ala
405 410 415

Ala Pro Pro Ala Lys Ala Ala Ala Pro Pro Ala Lys Ala Ala Ala Ala
420 425 430

Pro Val Gly Lys Lys Ala Gly Gly Lys Lys
435 440

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala
1 5 10 15

Ala Thr Ala Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala
1 5 10 15

Ala Ala Ala Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro
1 5 10 15

Ser Pro Phe Gly Gln Ala
20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala
1 5 10 15

Ala Ala Ala Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Lys Ala Ala Thr Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala Lys Ala
1 5 10 15

Ala Thr Ala Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ile Ala Pro Ala Lys Ala
1 5 10 15

Ala Ile Ala Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro
1 5 10 15

Ser Pro Phe Gly Gln Ala Gly Cys Gly Ser Ser Met Pro Ser Gly Thr
20 25 30

Ser Glu Glu Gly Ser Arg Gly Gly Ser Ser Met Pro Ala Gly Cys Gly
35 40 45

Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Gly Cys
50 55 60

Gly Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser
65 70 75 80

Gly Cys Gly

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Lys Thr Ala Ala Pro Pro Ala Lys Thr Ala Ala Pro Pro Ala Lys Thr
1 5 10 15

Ala Ala Pro Pro Ala
20

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 618 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Lys Ala Val Asp Pro Phe Gln Gly Thr Thr Pro Pro Pro Tyr Lys Trp
1 5 10 15

Gln Glu Met Thr Gly Ser Glu Ala Ala Ala Gly Ser Leu Cys Val Pro
20 25 30

Ser Leu Ala Glu Val Ala Gly Gly Val Phe Ala Val Ala Glu Ala Gln
35 40 45

Arg Ser Glu Arg Asp Glu Ala Cys Gly His Ala Ala Ile Ala Thr Thr
50 55 60

His Ile Glu Thr Gly Gly Gly Gly Ser Lys Ala Ile Ser Ala Met Asp
65 70 75 80

Ala Gly Val Phe Leu Val Glu Leu Val Asp Ala Ala Ser Gly Thr Ile
85 90 95

Arg Thr Arg Glu Lys Met Gln Pro Thr Thr Ile Val Ser Gly Asp Thr
100 105 110

Ile Tyr Met Ala Leu Gly Asp Tyr Glu Lys Lys Thr Ser Gly Gly Arg
115 120 125

Ala Ala Asp Ala Asp Gly Trp Arg Leu Leu Leu Met Arg Gly Thr Leu
130 135 140

Thr Glu Asp Gly Gly Gln Lys Lys Ile Met Trp Gly Asp Ile Arg Ala
145 150 155 160

Val Asp Pro Val Ala Ile Gly Leu Thr Gln Phe Leu Lys Arg Val Ile
165 170 175

Gly Gly Gly Gly Ser Gly Val Val Thr Lys Asn Gly Tyr Leu Val Leu
180 185 190

Pro Met Gln Ala Val Glu Lys Asp Gly Arg Ser Val Val Leu Ser Met
195 200 205

Arg Phe Asn Met Arg Ile Glu Ala Cys Glu Leu Ser Ser Gly Thr Thr
210 215 220

Gly Ser Asn Cys Lys Glu Pro Ser Ile Ala Asn Leu Glu Gly Asn Leu
225 230 235 240

Ile Leu Ile Thr Ser Cys Ala Ala Gly Tyr Tyr Glu Val Phe Arg Ser
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Leu Asp Ser Gly Thr Ser Trp Glu Met Ser Gly Arg Pro Ile Ser Arg
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Val Trp Gly Asn Ser Tyr Gly Arg Lys Gly Tyr Gly Val Arg Cys Gly
275 280 285

Leu Thr Thr Val Thr Ile Glu Gly Arg Glu Val Leu Leu Val Thr Thr
290 295 300

Pro Val Tyr Leu Glu Glu Lys Asn Gly Arg Gly Arg Leu His Leu Trp
305 310 315 320

Val Thr Asp Gly Ala Arg Val His Asp Ala Gly Pro Ile Ser Asp Ala
325 330 335

Ala Asp Asp Ala Ala Ala Ser Ser Leu Leu Tyr Ser Ser Gly Gly Asn
340 345 350

Leu Ile Ser Leu Tyr Glu Asn Lys Ser Glu Gly Ser Tyr Gly Leu Val
355 360 365

Ala Val His Val Thr Thr Gln Leu Glu Arg Ile Lys Thr Val Leu Lys
370 375 380

Arg Trp Gln Glu Leu Asp Glu Ala Leu Arg Thr Cys Arg Ser Thr Ala
385 390 395 400

Thr Ile Asp Pro Val Arg Arg Gly Met Cys Ile Arg Pro Ile Leu Thr
405 410 415

Asp Gly Leu Val Gly Tyr Leu Ser Gly Leu Ser Thr Gly Ser Glu Trp
420 425 430

Met Asp Glu Tyr Leu Cys Val Asn Ala Thr Val His Gly Thr Val Arg
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Gly Phe Ser Asn Gly Val Thr Phe Glu Gly Pro Gly Ala Gly Ala Gly
450 455 460

Trp Pro Val Ala Arg Ser Gly Gln Asn Gln Pro Tyr His Phe Leu His
465 470 475 480

Lys Thr Phe Thr Leu Val Val Met Ala Val Ile His Asp Arg Pro Lys
485 490 495

Lys Arg Thr Pro Ile Pro Leu Ile Arg Val Val Met Asp Asp Asn Asp
500 505 510

Lys Thr Val Leu Phe Gly Val Phe Tyr Thr His Asp Gly Arg Trp Met
515 520 525

Thr Val Ile His Ser Gly Gly Arg Gln Ile Leu Ser Thr Gly Trp Asp
530 535 540

Pro Glu Lys Pro Cys Gln Val Val Leu Arg His Asp Thr Gly His Trp
545 550 555 560

Asp Phe Tyr Val Asn Ala Arg Lys Ala Tyr Phe Gly Thr Tyr Lys Gly
565 570 575

Leu Phe Ser Lys Gln Thr Val Phe His Thr Ser Asn Ser Thr Gly Arg
580 585 590

Val Gly Lys Leu Gln Ser Pro Ala Ile Cys His Ser Ser Thr Pro Val
595 600 605

Cys Ile Thr Glu Asp Ser Ile Pro Ser Ile
610 615

Claims

1. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

2. The method of claim 1 wherein the biological sample is selected from the group consisting of blood, serum, plasma, saliva, cerebrospinal fluid and urine.

3. The method of claim 1 wherein the polypeptide is bound to a solid support.

4. The method of claim 3 wherein the solid support comprises nitrocellulose, latex or a plastic material.

5. The method of claim 3 wherein the step of detecting comprises:

(a) removing unbound sample from the solid support;

(b) adding a detection reagent to the solid support; and

(c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, and therefrom detecting *T. cruzi* infection in the biological sample.

6. The method of claim 5 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

7. The method of claim 6 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G, Protein A and lectins.

8. The method of claim 6 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

9. A polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:21, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

10. An isolated DNA sequence encoding a polypeptide according to claim 9.

11. A recombinant expression vector comprising a DNA sequence according to claim 10.

12. A host cell transformed or transfected with an expression vector according to claim 11.

13. The host cell of claim 12 wherein the host cell is selected from the group consisting of *E. coli*, yeast, insect cell lines and mammalian cell lines.

14. A diagnostic kit for detecting *T. cruzi* infection in a biological sample, comprising:

(a) a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications: and

(b) a detection reagent.

15. The kit of claim 14 wherein the polypeptide is bound to a solid support.

16. The kit of claim 15 wherein the solid support comprises nitrocellulose, latex or a plastic material.

17. The kit of claim 14 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

18. The kit of claim 17 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G , Protein A and lectins.

19. The kit of claim 17 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

20. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting a biological sample with a monoclonal antibody that binds to an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) detecting in the biological sample the presence of *T. cruzi* parasites that bind to the monoclonal antibody, therefrom detecting *T. cruzi* infection in the biological sample.

21. The method of claim 20, wherein the monoclonal antibody is bound to a solid support.

22. The method of claim 21 wherein the step of detecting comprises:

(a) removing unbound sample from the solid support;

(b) adding a detection reagent to the solid support; and

(c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, therefrom detecting *T. cruzi* infection in the biological sample.

23. The method of claim 22 wherein the detection reagent comprises a reporter group coupled to an antibody.

24. A pharmaceutical composition comprising:

(a) a polypeptide, wherein the polypeptide comprises an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) a physiologically acceptable carrier.

25. A vaccine for stimulating the production of antibodies that bind to *T. cruzi*, comprising:

(a) a polypeptide, wherein the polypeptide comprises an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) an adjuvant.

26. A method for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a pharmaceutical composition according to claim 24.

27. A method for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a vaccine according to claim 25.

28. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

29. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications;

(c) contacting the biological sample with a third polypeptide comprising an epitope of TcE, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(d) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

30. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications;

(c) contacting the biological sample with a third polypeptide comprising an epitope of PEP-2, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(d) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

31. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcE, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

32. The method of claim 31 wherein the second polypeptide comprises the amino acid sequence Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala (SEQ ID NO:56).

33. A combination polypeptide comprising two or more polypeptides according to claim 9.

34. A combination polypeptide comprising at least one epitope of a T. cruzi antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22. or a variant of said antigen that differs only in conservative substitutions and/or modifications. and at least one epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications.

35. A combination polypeptide according to claim 34, wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:55-56.

36. A combination polypeptide according to claim 34, wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:53-54.

37. A combination polypeptide according to claim wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:57.

38. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a combination polypeptide according to any one of claims 33-37; and

(b) detecting in the biological sample the presence of antibodies that bind to the combination polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

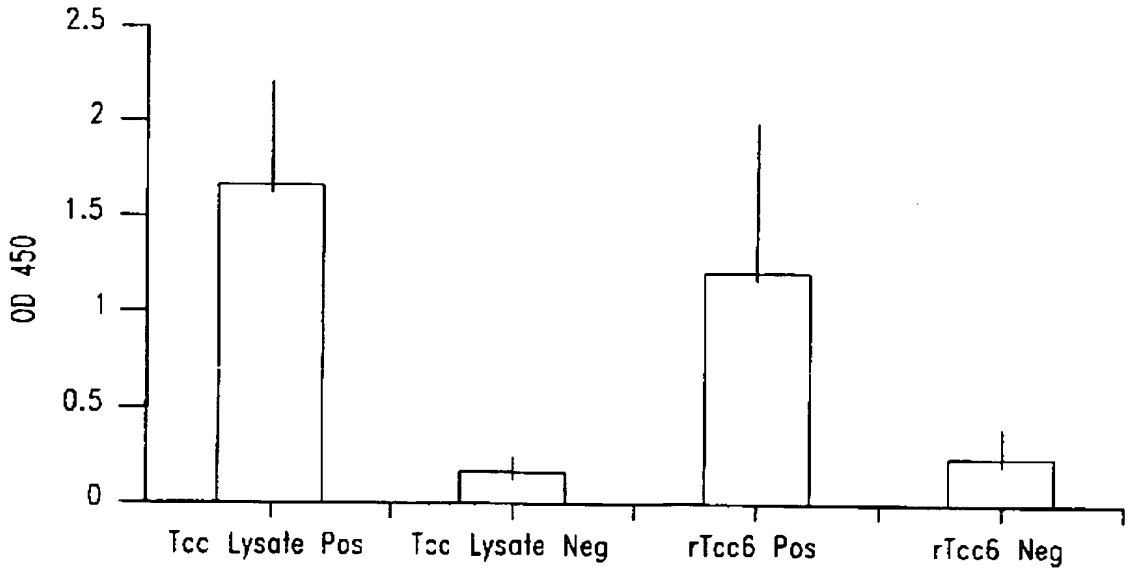

Fig. 1

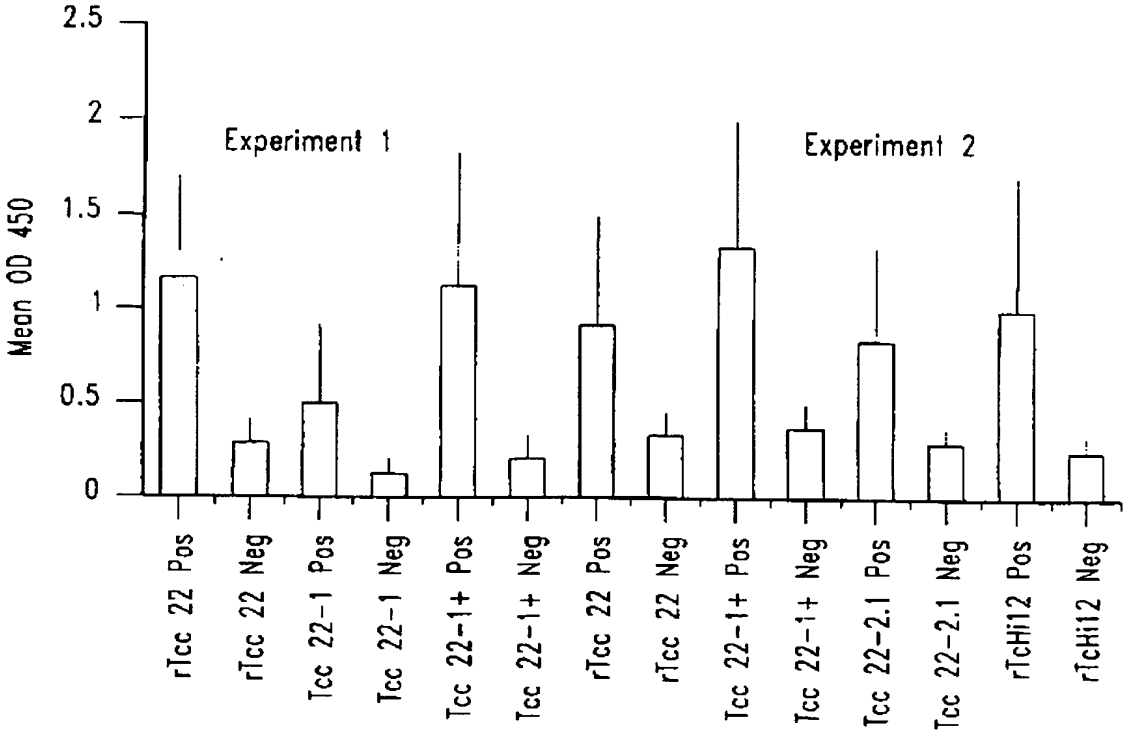

Fig. 2

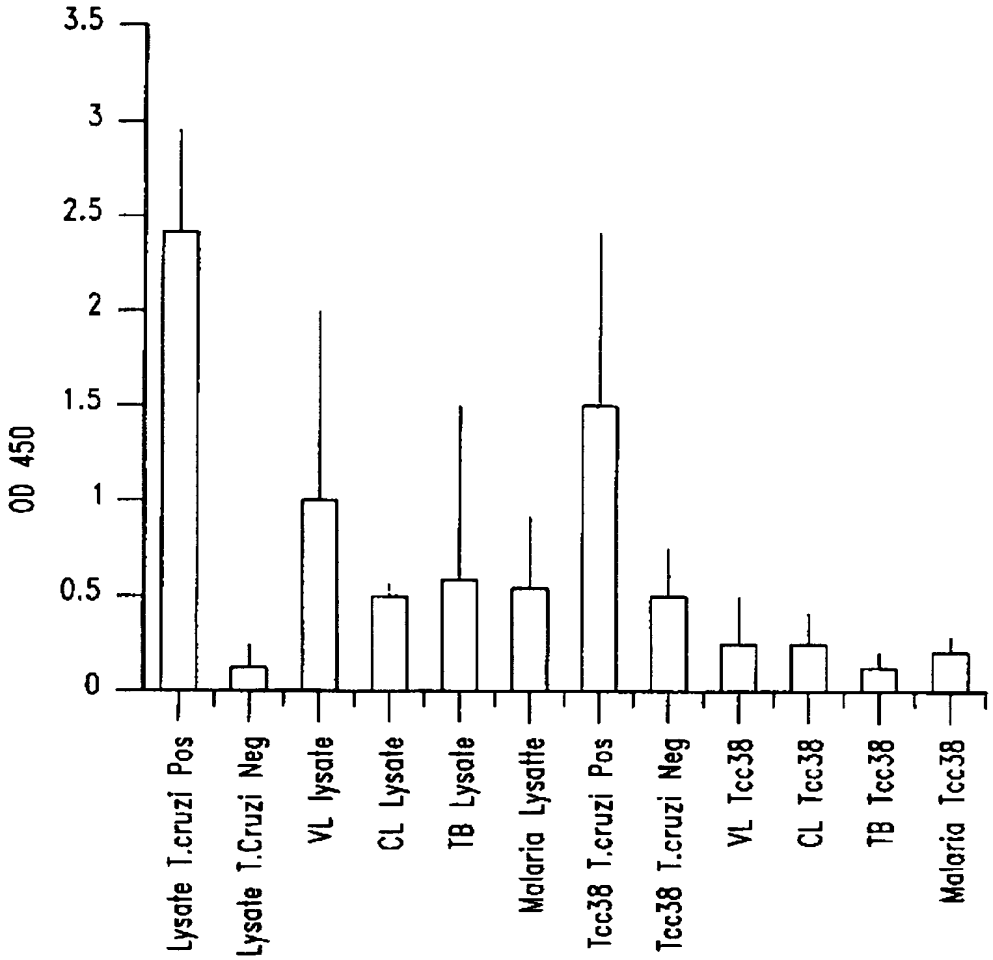

Fig. 3

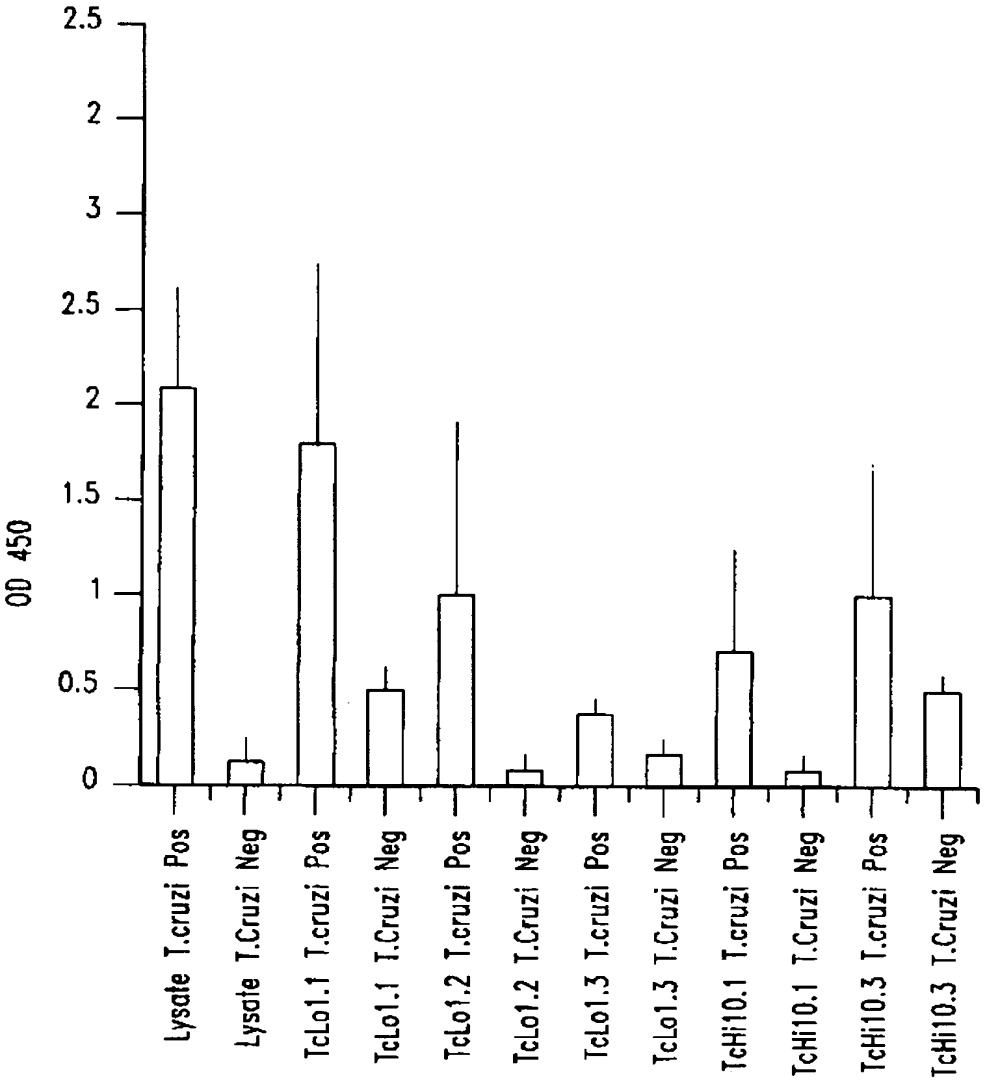

Fig. 4

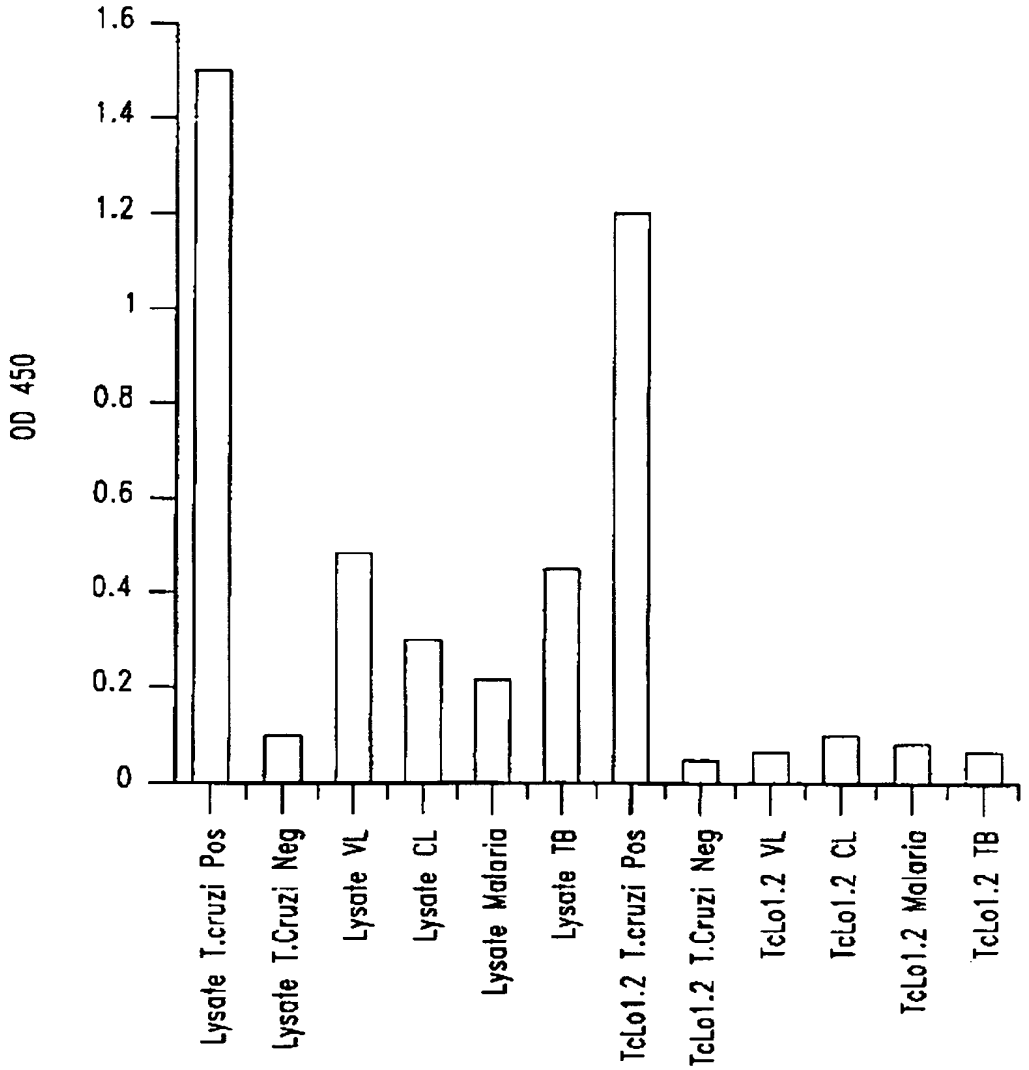

Fig. 5

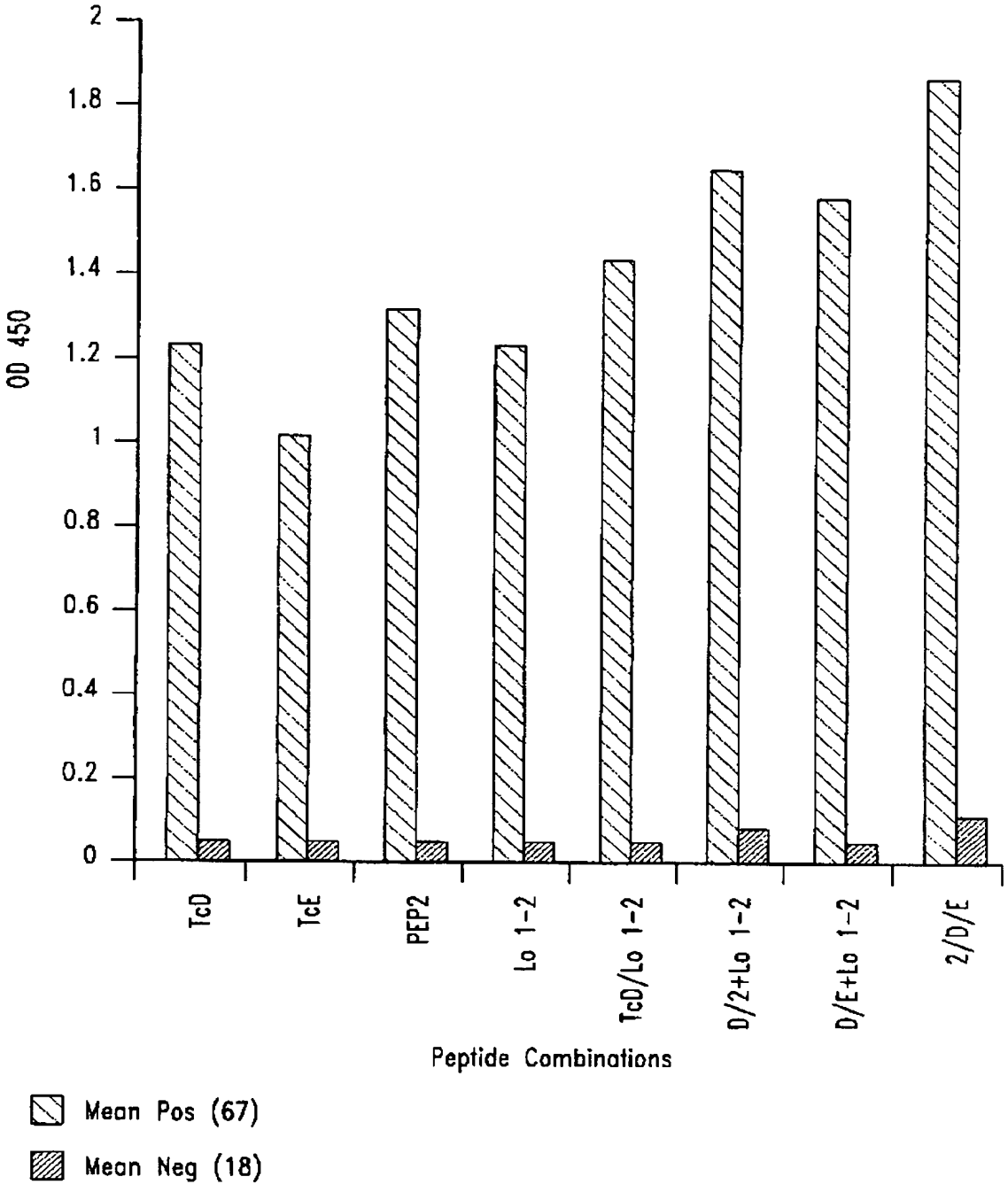

Fig. 6

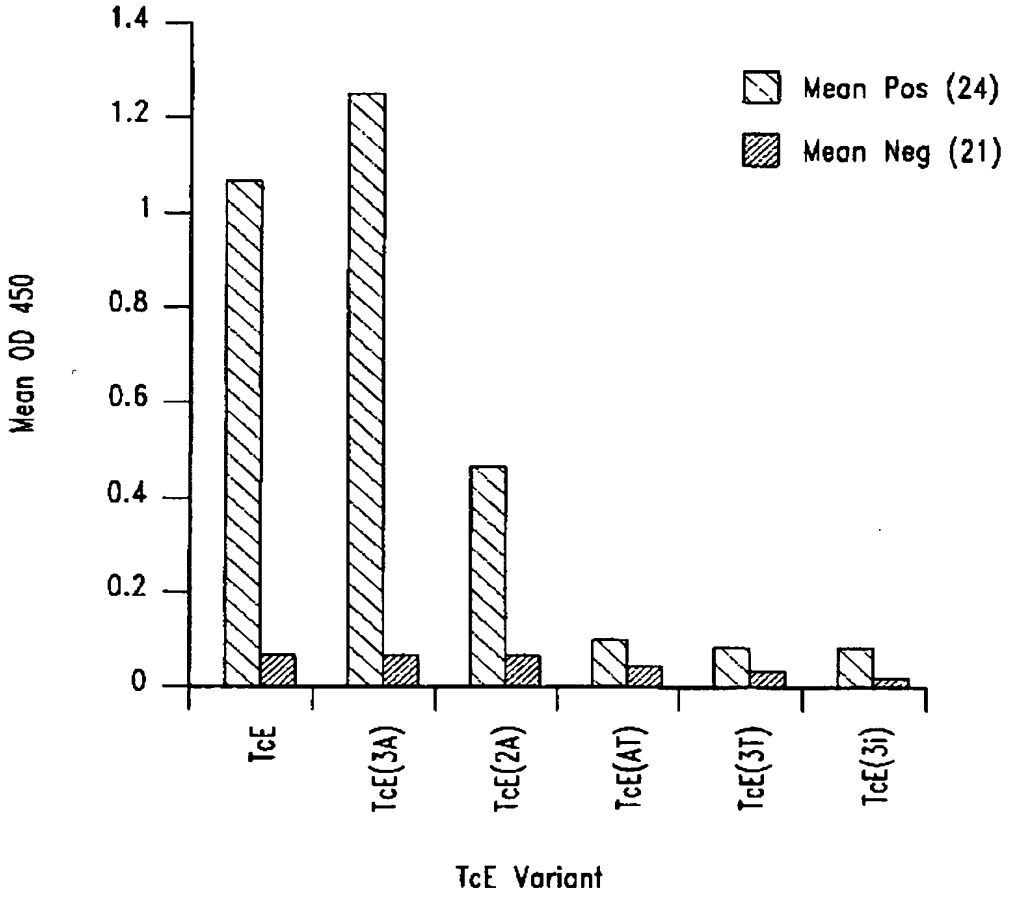

Fig. 7

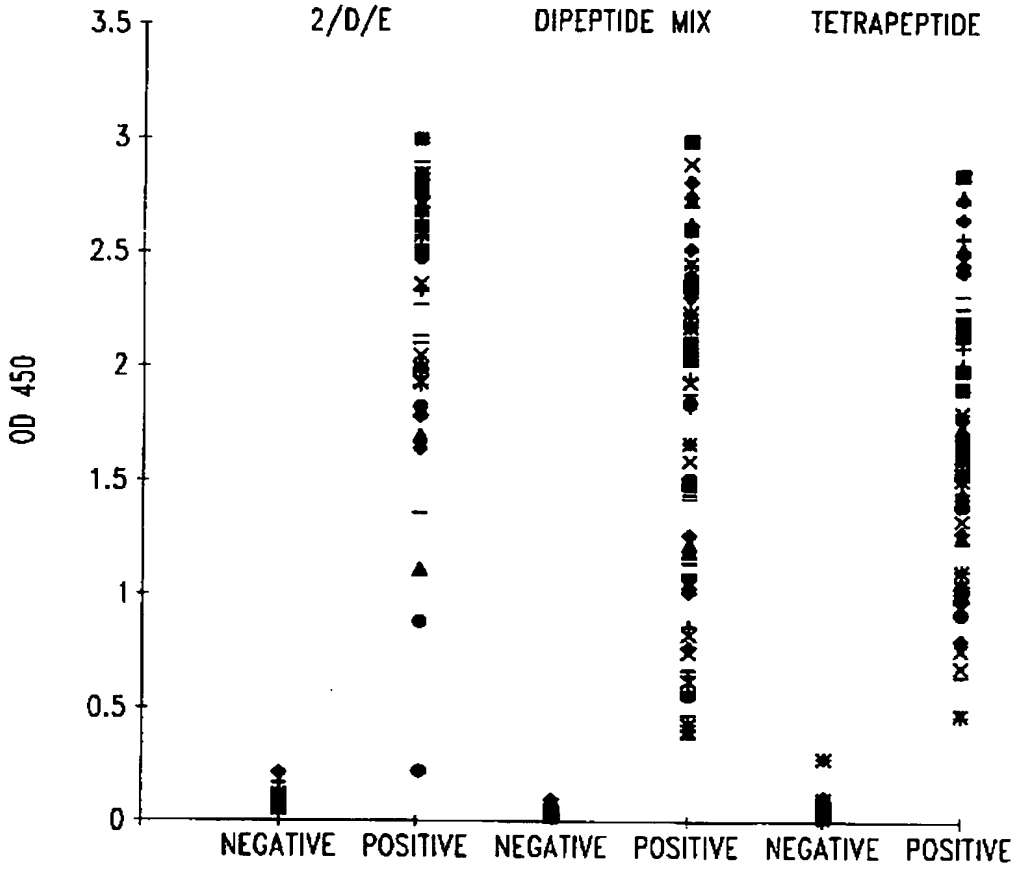

Fig. 8

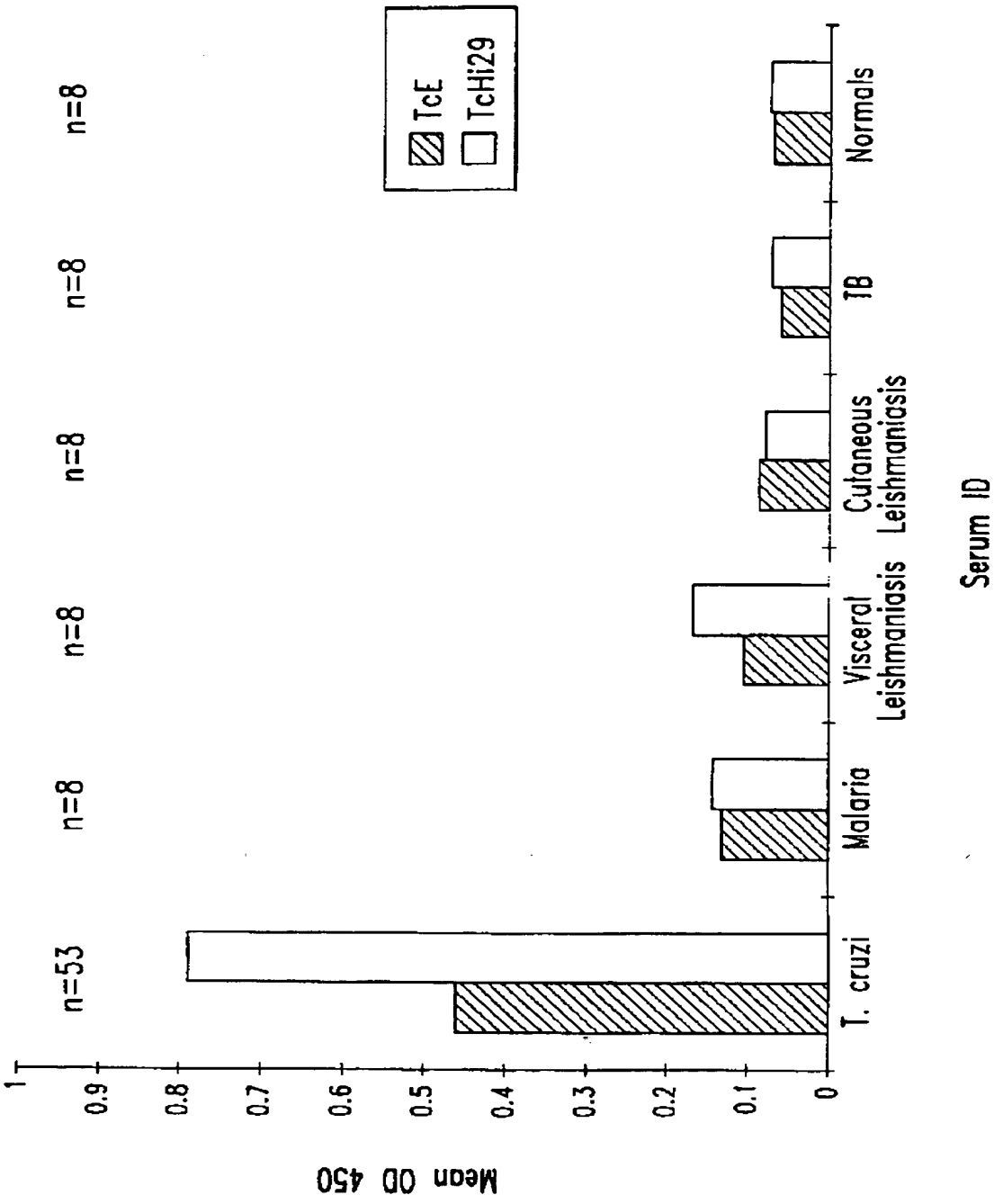

Fig. 9

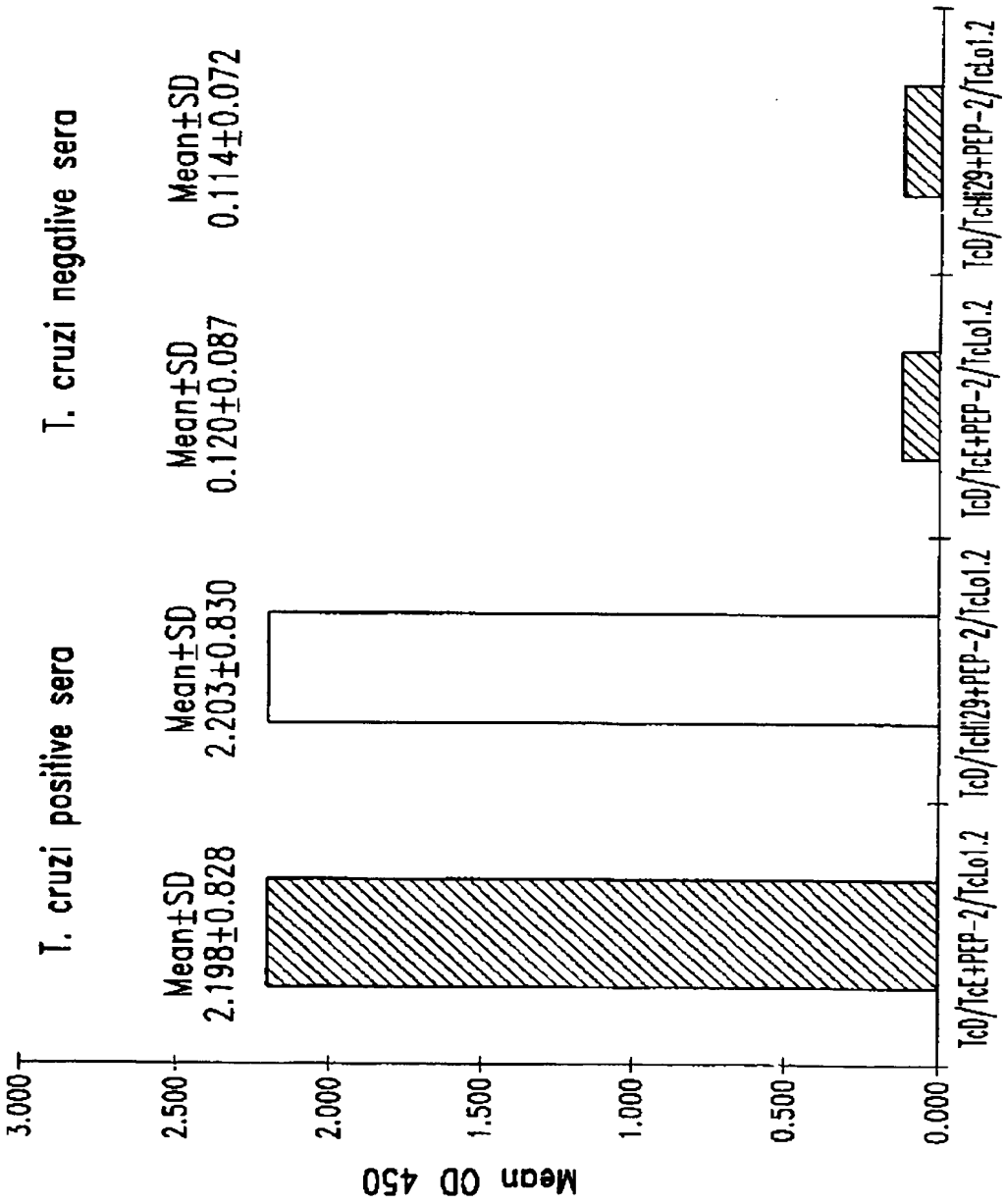

Fig. 10

INTERNATIONAL SEARCH REPORT

Inter [redacted]nal Application No
PC[redacted]/US [redacted]8624

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/569 C07K14/44 C12N15/12 C12N15/85 C12N1/21
A61K39/005 G01N33/543 A61K39/002 A61K39/008

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CLINICAL MICROBIOLOGY, vol. 32, no. 4, 1 April 1994, WASHINGTON DC USA, XP000603777 J.M. PERALTA ET AL.: "Serodiagnosis of Chagas' disease by enzyme-linked immunosorbent assay using two synthetic peptides as antigens." see the whole document ---	1-38
A	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 176, no. 1, 1992, NEW YORK NY USA, pages 201-211, XP000603651 Y.A.W. SKEIKY ET AL.: "Cloning and expression of Trypanosoma cruzi ribosomal protein P0 and epitope analysis of anti-P0 autoantibodies in Chagas' disease patients." see the whole document --- -/--	1-38

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 1997	05 -03- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Van Bohemen, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY, vol. 151, no. 10, 15 November 1993, WASHINGTON DC USA, pages 5504-5515, XP000604843 Y.A.W. SKEIKY ET AL.: "Trypanosoma cruzi acidic ribosomal P protein gene family" see the whole document ---	1-38
A	INFECTION AND IMMUNITY, vol. 62, no. 5, 1 May 1994, CHICAGO IL USA, pages 1643-1656, XP000604822 Y.A.W. SKEIKY ET AL.: "Antigens shared by Leishmania species and Trypanosoma cruzi: immunological comparison of the acidic ribosomal P0 proteins." see the whole document ---	1-38
A	US 5 304 371 A (S.G. REED) 19 April 1994 cited in the application see the whole document ---	1-38
A	WO 93 16199 A (S.G. REED) 19 August 1993 see the whole document ---	1-38
A,P	WO 96 29605 A (CORIXA CORPORATION) 26 September 1996 see the whole document -----	1-38

1

INTERNATIONAL SEARCH REPORT

Information [redacted]ent family members

Inter[redacted]nal Application No
PC[redacted]/US[redacted]8624

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US 5304371 A	19-04-94	CA 2129747 A	15-08-93
		EP 0649475 A	26-04-95
		WO 9316199 A	19-08-93
		US 5413912 A	09-05-95
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		CA 2129747 A	15-08-93
		EP 0649475 A	26-04-95
		US 5413912 A	09-05-95
WO 9629605 A	26-09-96	AU 5362696 A	08-10-96

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